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Molecular Characterization of Fusion and Glycoprotein Genes of RSV Genotype a in Infants Suffering From Influenza Like Symptoms in Iraq

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ABSTRACT

Respiratory syncytial virus (RSV) is one of the most common respiratory viruses that causes bronchiolitis in infant all over the world. Differentiation of the contiguous genotype A and B of this virus in children less than five years old with influenza like illness (ILI) was carried out by real-time RT-PCR targeting the Fusion (F) and Glycoprotein (G) genes. Results showed that 31% of children with ILI were infected with RSV type A while no genotype B was found in any of the patients tested. Targeting partial sequence of F gene of RSV using nested RT-PCR revealed positive amplicons of 90 bp in twenty specimens. Similarly, targeting a partial sequence of the G gene of RSV revealed positive amplicons of 391bp for two isolates only in the second round of amplification. Phylogenetic analyses were conducted for the partial sequences of F and G genes type a using MEGA 6. Results showed that strain A2 was found among 61% of specimens tested for F gene. Sequencing and phylogenetic tree analyses of the G gene showed similarity to the two novel genotype of RSV type A (GA2 and ON1) circulating in children under five years old in Iraq.

Key words: *RSV, Fusion Gene, Glycoprotein Gene, RT-PCR, Nested PCR*

1. INTRODUCTION

Respiratory syncytial virus (RSV) is one of the highly contiguous viruses that cause a severe illness in infants under five years old of age worldwide. RSV is one the most common pathogens causing bronchiolitis, pneumonia, and chronic obstructive pulmonary infections (Feshbach et al., 2013), leading to 160,000–600,000 deaths per year in children under 5 years of age (Krilov, 2011). RSV is a member of the family *Paramyxoviridae* with non-segmented negative-strand RNA. The viral genome is about 15,000bp and it consists of 10 genes that are transcribed to 11 proteins (Collins and Crowe, 2006).). The most important virus surface proteins that play a role in the pathogenesis and host immune response, are the small hydrophobic (SH), the attachment Glycoprotein (G) and the fusion (F) protein. The G glycoprotein is involved in the viral attachment and used for RSV genotyping because of its variable characterization that might contribute to the ability of the virus to cause annual outbreaks (Sullender, 2000; Yamaguchi et al., 2011). The F protein of RSV is considered a conserved region that mediates the fusion process and plays a major role in viral attachment, as well as it induces the innate immune response (Zlateva et al., 2005). RSV strains are separated into two major groups A and B, depending on sequence differences found in the G gene. RSV-A genotypes are designated for ten types GA1 to GA7, SAA1 (Peret et al., 1998 and 2000), NA1 and NA2 (Venter et al., 2001). On the other hand, RSV-B genotype is distinguished as GB1 to GB4, SAB1 to SAB3, and BA1 to BA6 (Shobugawa et al., 2009). In this study, the prevalence of genotypes A and B of RSV were investigated to determine their role in ILI in children younger than 5 years old in Iraq. Sequencing and phylogenetic analysis of the F and G genes were carried out to underpin the specific genotypes circulating in children in Iraq.

2. MATERIALS AND METHODS

2.1. Clinical samples

Nasal and throat swabs were collected during late autumn and winter of 2014 from one hundred of outpatient infants, their age ranged from two months up to five years old. All patients suffered from influenza like illness (ILI) according to WHO guideline (2006). Symptoms including running nose, fever and wheezing chest were defined with physical examination by the consultant physicians and symptoms conformed by chest X ray.

2.2. Real-Time PCR (qRT- PCR) targeting Glycoprotein gene (G)

Extraction of RNA was performed using QIAamp Viral RNA Mini spin protocol following the manufacturer's instructions (Qiagen, Hilden, Germany). cDNA was generated by reverse transcriptase reaction targeting G gene, using one step real-

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time quantitative RT-PCR (q-RT-PCR). Primer pairs were designed and synthesized by Primer Design, England and qRT-PCR Applied Biosystem 7500 machine. Primers and labeled hydrolysis (Taqman ®) probes for RSV genotype A and B were used to specifically detect RSV in patients' samples for 50 cycles, using amplification conditions which as follows: 10 min. at 55 °C for reverse transcriptase, 8 min at 95 °C for enzyme activation, 10 sec at 95 °C for denaturation and 60 sec at 60 C °C for data collection.

2.3. qRT-PCR targeting Fusion gene (F)

RT-PCR targeting F gene was performed using QIAGEN One Step RT-PCR (Qiagen, Hilden, Germany), oligonucleotide primers and Taqman probes 6-FAM/TAMRA (10, 11). Primers used targeting genotype A were; forward primer A,`5-AACAGATGTAAG CAGCTCCGTTATC-3`, reveres primer A,`5-CGATTTTTATTGGATGCTGTACATTT-3`. Primers used targeting genotype B were; forward primer B,`5-GGAAACATACGTGAACAAGCTTCA-3`, Reveres Primer B,`5-TCATCATCTTTTTCTAGAACATTGTACTGA-3`. Probe sequence was; `5-TGGCATAGCATGACACAATGGCTCCT-3`. RT-PCR Applied Biosystem-7500 was used to run RSV samples for 50 cycles. Amplification conditions of qRT-PCR targeting partial sequence of F gene of RSV is as previously described (Mentel et al., 2003).

2.4. Conventional RT-PCR targeting partial sequence of F gene

RT-PCR was conducted using oligonucleotides primers for RSV type A as previously described (Mentel et al., 2003). The reaction was done in a final volume of 50µl reaction mixture with 5-l µl of cDNA and 45µl of PCR mixture with Dream Taq Green 2X. Thermal cycling parameters included, 40 cycles of denaturation at 95°C for 30sec, annealing 50°C for 30sec, 72°C for 1 min extension stage and 72°C for 10 min for final extension (Mentel et al., 2003).

2.5. Nested RT-PCR targeting partial sequence of G gene

A representative number of high and moderate Ct value type A RSV-positive samples were selected for amplification of hyper variable region of the G gene by nested PCR. The first amplification was performed with 5µl cDNA in a 50µl reaction mixture by using 250 pmol of each of the primers; RSVA-G513-F and RSVA-G131-R Dream Taq Green 2X. Primers used for the amplification of partial sequence of G gene are previously described (Reiche and Schweiger, 2009). Gene amplification was carried out at 94°C for 5 min, followed by 40 cycles of PCR, with 1 cycle consisting of 30 sec at 94°C, 30 sec at 58°C, and 1 min at 72°C, and a final extension step of 10 min at 72°C. The second run of nested PCR carried out with the negative results, 5 µl of the external PCR mixture was used for nested PCR, which was performed in a 50µl reaction mixture with 250 nM of RSVA-G606-F and RSV-F22-R (see Table 3). The cycling protocol was the same as for the external PCR, except for the annealing temperature, which was optimized to 53°C (Reiche and Schweiger, 2009).

2.6. Agarose gel electrophoresis.

The DNA bands of RSV were determined using gel electrophoresis technique using 1-1.5% agarose gel concentration (Sambrook et al., 1989). PCR products were visualized on an ethidium bromide-stained agarose gel using a UV transilluminator. A 50bp and 1kb marker was used to control fragment lengths.

2.7. DNA Extraction and sequencing

RT-PCR products of both F and G genes were extracted either directly from PCR samples or eluted from an agarose gel, with QIAquick PCR extraction kit according to the manufacturer's instructions. Both strands were sequenced on a Genetic analyzer 3130xl (Applied Biosystem, USA), using a fluorescent dye terminator and BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystem, USA).

2.8. Analysis of sequences

Raw sequencing data were processed by the ABI Sequence Analysis Programme 5.1.1 (Applied Biosystems, Foster City, USA) then edited, assembled and compared using the STADEN Package version 2003.0 Pregap4 and Gap4 Programs (Bonfield et al., 1995). The sequences were compared to published sequencing data in GenBank (National Center for Biotechnology Information, Bethesda, USA) online (www.ncbi.nih.gov) using BLASTN and BLASTX options. Multiple alignments of nucleotide sequences were performed with ClustalW algorithm of the BioEdit Sequence Alignment Editor program (Hall, 1999). This alignment was further analysed for phylogenetic calculations in the MEGA 6 software program comparing neighbor-joining method. Bootstrap analysis of 500 replicates was carried out.

3. RESULTS AND DISCUSSION

3.1. Genotyping of RSV A and B

QRT-PCR targeting partial sequence of F and G genes revealed RSV genotype A among 31% of patients tested. The Ct values of the positive samples were 16 – 35.8 (data not shown). The susceptible ages that showed the highest infection rate of RSV in children ranged between 2 months to 2 years as summarized in Table (1). No RSV genotype B was detected in samples tested. Results showed positive amplicons in twenty specimens of RSV genotype A targeting partial sequence of F gene with high and moderate Ct value. The expected RT-PCR specific product size is 90 bp. Two isolates out of twenty were positive for the RT-PCR targeting partial sequence of the G gene. The first isolate was given code No.129 with expected size product of 583bp in the first round of the nested PCR. The second isolate was given the

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No.154 with expected size of 391bp as second round products.

3.2. Phylogenetic and genotyping analyses of RSV genotype A

Phylogenetic tree analysis with MEGA 6 using several options (Maximum Likelihood analyses, Minimum Evolution Method, Neighbor Joining Method) was conducted based on partial sequence analyses of the 90 bp amplified F gene from six RSV. All six RSV positive specimens (2770, 2977, 148, 147, 152 and 2980) were shown to be clustered within human RSV strain A lineage. Multiple sequences alignment with ClustalW together with phylogenetic evolution tree analyses showed that the RSV A local isolates were clustered into two groups compared to those for the NCBI references strains Figure (4). This segregation formed a significant clade of two groups. Isolates no. 147, 148 and 2770 clustered together and showed 100% nucleotide identity value. Isolates No. 152, 2977, 2880 were segregated alone in two subclusters and showed bootstrap value of 99%. Interestingly, this segregation is still closer to genotype A but positioned between the two genotypes A and B (Figure 1).

3.3. Phylogenetic analysis for RSV genotype A for G gene

The nucleotides sequence of the RSV A local isolates 129 and 154 were analyzed by the NCBI BLAST and showed high similarity with the human RSV genotype A (Figure 2). The local isolate No. 129 showed identity ranged from 99% to 96% with the novel RSV genotype ON1 whereas isolate No. 154 showed identity percentage of 99% to 100% with the novel genotype GA2.

Multiple sequences alignments of the partial G gene sequence with 20 reference strains using BioEdit and MEGA 6, results showed high homology in nucleotides sequence. Phylogentic analysis using distance Maximum likelihood tree showed bootstrap value of 64-100%, bootstrap value less than 60% was not shown in the tree (16). The local RSV isolate No. 129 clustered with a strain that was previously assigned to the novel ON1 genotype with a72-nt duplication GenBank accession No. (*KJ710402.1)* HRSV. In addition, the second isolate No. 154 was clustered with a strain of GA2 GenBank accession No. (*KJ6724402.1*-HRSV) (Figure 2).

RSV is one of the most important respiratory viruses causes bronchiolitis infection among infants worldwide. In this study, the genotype prevalence of RSV in young children clearly indicated that this virus constitutes a serious problem in causing ILI among Iraqi children of age range from 2 months to 2 years. These findings are consistent with previously published work (Tran et al., 2012) describing the dominant genotype A of seasonal RSV infection. The prevalence of this infection was peaked during the winter season between December to February 2014. This observation is in agreement with other previously reported study (Murray et al., 2012). This may explain in part the association of RSV with the acute respiratory infection during winter. However, comparing these positive results with international rate of infection with RSV, it may be considered high with a rate of 31%. The international rate ranged between 14% - 34% for RSV (Nair et al., 2010; CDC, 2013) and 14 per 100,000 in children less than 5 years old (CDC, 2012). These differences could be attributed to variabilities in the length of infection in winter season which usually last for up to six months or due to other factors such as differences of environmental and host factors that may influence seasonal virus transmission and severity of infection (Martinello et al., 2002). However, it is not yet clear why and how different genotypes evolved and circulates in population and how they influence virulence. On the other hand, the rate of infection in this study was reported by other studies in low- and middle-income countries (Marika et al., 2013).

Acute respiratory infection in Iraq showed a mortality rate of 16% in children under 5 years old (WHO, 2013). It was also suggested that RSV genotype A contributes up to 72% more frequently than RSV-B (John, 2004). Nevertheless, a recent study of RSV infection in north Iraq in Sulaymaniya, showed that RSV rate of infection among children less than 5 years old was 27% (Hussain, 2013). However, both RSV genotype A and B were identified with infection rate 77% and 22% respectively. Results in this study contradicts those findings in the north of the country where genotype B was absent in all patients tested in Baghdad city. It was noted that a limited variability among the group B viruses might contribute to a more restricted spread and circulation of RSV in certain regions. This eventually may lead to the dominance of group A over group B isolates in many epidemiological studies of RSV (Shobugawa et al., 2009).

Phylogenetic analysis for partial sequence of F and G genes of RSV showed that 61% of the RSV local isolates were classified as RSV A genotype. The frequency of A2 strain in this study was 10%. This proportion is consistent with a previous study (14). The importance of the F gene lies in its role as a target for drug and vaccine development (Tapia et al., 2014). The G gene was used for subtyping in many previous studies (Tabatabai et al., 2014). Data showed that G gene in RSV genotype A has been classified into GA1–GA7 and SAA1 genotypes GA1 strain. The latter is considered one of the most virulent subtypes (Peter and Graham, 2008). Therefore primers used in this study in nested PCR experiments were derived targeting RSV genotype A strain A2, GenBank Accession No. (*U50362.1)*. This is because RSV genotype A strain A2 was shown in a previous study to be associated with a more-severe disease (Galiano et al., 2005).

Partial sequence analysis of F gene showed high genetic diversity within RSV genotype-A which may suggest that new genotypes may be emerging over time which is aligned with previous studies (Hsin et al., 2013). Interestingly, sequence and phylogenetic analyses of F gene showed these

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local isolates diverged away from RSVA otherwise were placed in a transitional stage between RSV A and B. On the other hand, RSV evolutionary divergences were in continuous progresses due to differences in selective pressure in a distinct pattern or due to the immune protection of the hosts. The results here are in agreement with (Fernanda et al., 2014) which indicate that multiple genotypes may co-circulate in a single epidemic and that the genotypes in each epidemic may differ. These results are also in consistent with a study in Taiwan (Hsin et al., 2013) which demonstrated that RSV isolates showed significant faster evolution after 2005. The dominant subtypes of RSV-A genotype in each epidemic were replaced by different subtypes in the subsequent epidemic. The present results also were in agreement with previous studies of RSV F gene that showed that newly generated nucleotides sequences isolates from children in Canada have emerged during 2008–2009 by 44% and became the predominant RSV-A clade within a year during 2009– 2010 100% (Papenburg, et al., 2012). Nevertheless, although these mutant strains are newly spread worldwide, but the phylogenetic ancestors analysis for the RSV A local isolates showed that RSV ancestors may be originated from mutant strains with GenBank accession *M11486.1* RSHICE: Human RSV accession *JF920069.1* HRSV A/W1629-3/06-07, accession *U50363.1* Human RSV mutant cp-RSV, accession U50362.1 Human RSV mutant cp-RSV which was compared with strains obtained from NCBI blast results.

The ancestor assignment targeting G gene in the two RSV local isolates by ML analysis showed that *KJ672446.1* RSV/Homo sapiens/ USA/LA2 27/2012 may assume to be the most likely close resemblance. On the other hand, the RSV local isolates that found similar to RSV-A genotype ON1 was reported in different studies from Asia, Africa and Europe suggesting a worldwide emergence of the novel RSV-A strain.

During the winter seasons in 2010 and 2011, a novel RSV-A genotype ON1 with 72-nt duplication has been reported in Canada (Eshaghi et al., 2012). In Beijing, China, 2012 only one sample out of about 250 RSV-A sequenced was characterized as ON1 genotype. Reports about circulating genotypes in the season 2012–2013 were published from Cyprus, Italy, Kenya and South Korea described ON1 as the predominating genotype in the epidemic season 2012–2013 (Pierangeli et al., 2014). Similarly, results here agree with these investigations, although with less number of isolates (5%). This percentage was found to be higher in Germany 10% (Prifert et al., 2013). Again, results in this study also showed the presence of genotype GA2 in about 5% of the local RSV A isolates. It was also shown by previous studies that the existing predominant genotype can be replaced by a new genotype, as in the case of RSV-A genotypes GA2, GA5 and GA7 were replaced by NA1 and NA2, and BA became a predominant RSV-B genotype (Khor et al., 2013). It worth's noted that there is a lack of information about which circulated genotypes of RSV in Iraq. Therefore comparison of our results with the previous results for the same region is currently not doable and requires further investigation. These findings need to be verified with a larger number of isolates.

4. CONCLUSIONS

Results of this study showed that strain A2 of RSV was found among 61% of specimens tested for F gene. Sequencing and phylogenetic tree analyses of the G gene showed similarity to the two novel genotype of RSV type A (GA2 and ON1) circulating in children under five years old in Iraq. In conclusion more attention needs to be directed toward RSV genotypes and the novel variants which may influence or enhance the clinical severity of respiratory illnesses caused by these variants and could eventually serve in vaccine design regiments.

REFERENCES

Sullender WM. (2000). Respiratory syncytial virus genetic and antigenic diversity. Clin Microbiol Rev. 13(1): 1–15.

Melero, JA and Moore ML. (2013). Influence of Respiratory Syncytial Virus Strain Differences on Pathogenesis and Immunity. Curr Top Microbiol Immunol. 372: 59–82.

Feshbach K. Bloom-, Alonso W. J., Charu V. (2013). "Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): a global comparative review," PLoS ONE, vol. 8, no. 2, Article IDe54445/

Krilov L. R. (2011). "Respiratory syncytial virus disease: update on treatment and prevention," Expert Review of Anti-Infective Therapy, vol. 9, no. 1, pp. 27–32.

Collins PL, Crowe JE. (2006). Respiratory Syncytial Virus and Metapneumovirus. In: Knipe DM, Howley PM, editors. Fields Virology. Lippincott Williams & Wilkins. pp. 1601–1646.

Yamaguchi M, Sano Y, Dapat IC, Saito R, Suzuki Y, Kumaki A, Shobugawa Y, Dapat C, Uchiyama M, Suzuki H. (2011). High frequency of repeated infections due to emerging genotypes of human respiratory syncytial viruses among children during eight successive epidemic seasons in Japan. J Clin Microbiol. ;49:1034–1040. doi: 10.1128/JCM.02132-10.

Zlateva KT, Lemey P, Moes E, Vandamme AM, Van Ranst M. (2005). Genetic variability and molecular evolution of the human respiratory syncytial virus subgroup B attachment G protein. J Gen Virol 79: 9157–9167.

Peret TC, Hall CB, Schnabel KC, Golub JA, Anderson LJ. (1998). Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. J Gen Virol 79: 2221–2229.

Peret TC, Hall CB, Hammond GW, Piedra PA, Storch GA, et al. (2000). Circulation patterns of group A and B human respiratory syncytial virus genotypes in 5 communities in North America. J Infect Dis 181: 1891–1896.

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Venter M, Madhi SA, Tiemessen CT, Schoub BD. (2001). Genetic diversity and molecular epidemiology of respiratory syncytial virus over four consecutive seasons in South Africa: Identification of new subgroup A and B genotypes. J Gen Virol 82: 2117–2124.

Shobugawa Y, Saito R, Sano Y, Zaraket H, Suzuki Y, et al. (2009). Emerging genotypes of human respiratory syncytial virus subgroup A among patients in Japan. J Clin Microb 47: 2475–2482.

Mentel, R.; Wegner, U.; Bruns, R. and. Gurtler, L. (2003). Real-time PCR to improve the diagnosis of respiratory syncytial virus infection .J. of Medi. Microbio., 52: 893–896.

Kwofie, T.B.; Anane, Y.A.; Nkrumah, B.; Annan, A.; Nguah, S.B. and Owusu, M. (2012). Respiratory viruses in children hospitalized for acute lower respiratory tract infection in Ghana. *Virol J.*, 9**:**78.

Reiche, J. and Schweiger, B. (2009). Genetic variability of group A human respiratory syncytial virus strains circulating in Germany from 1998 to 2007. J Clin Microbiol., 47: 1800–1810.

Sambrook J, Fritschi EF and Maniatis T. (1989). Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York

Bonfield, J. K., Smith, K. F. and Staden, R. (1995). A new DNA sequence assembly program. Nu cleic Acids Research, 23(24), 4992- 4999.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 41, 95–98.

Abbas, M.D. (2013). Diagnosis of ferla viruses in snakes and characterization of isolates based on gene sequences. PhD. Thesis Univ. of Hohenheim. 58:71, 72.

Tran, D.N.; Pham, T.M.H.; Ha, M.T.; Tran, T.T.L.; Dang, T.K.H.; Yoshida, L.M.; Okitsu, S.; Hayakawa, S.; Mizuguchi, M. and Ushijima, H. (2013). Molecular Epidemiology and Disease Severity of Human Respiratory Syncytial Virus in Vietnam. PLoS ONE., 8(1): e45436.

Murray, E.L.; Klein, M.; Brondi, L.; McGowan ,J.E.; Van Mels, C.; Brooks, W.A.; Kleinbaum, D.; Goswami, D.; Ryan ,P.B. and Bridges, C.B. (2012). Rainfall, household crowding, and acute respiratory infections in the tropics. Epidemiol .Infect., 140:78-86.

Nair, H.; Nokes, D.J.; Gessner, B.D.; Dherani, M.; Madhi ,S.A.; Singleton, R.J.; O'Brien, K.L.; Roca, A.; Wright, P.F.; Bruce, N; Chandran ,A.; Theodoratou, E.; Sutanto, A.; Sedyaningsih, E.R.; Ngama, M.; Munywoki ,P.K.; Kartasasmita, C.; Simões, E.A; Rudan, I.; Weber, M.W. and Campbell, H. (2010). Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet., 375:1545-1555.

Central of disease control and prevention, (CDC). (2013) .Respiratory Syncytial Virus Activity --- United States, Morbidly and mortality weekly report (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6208a1.htm).

Centers for Disease Control and Prevention (CDC) Update. (2012). Influenza A (H3N2) transmission and guidelines - five states, 2011. MMWR Morb. Mortal. Wkly. Rep., 60: 1741–1744

Martinello, R.A.; Chen, M.D.; Weibel, C. and Kahn, J.S. (2002). Correlation between respiratory syncytial virus genotype and severity of illness. J Infect Dis., 186:839–842.

Marika, K. I. Eileen, C. F. and Susan I. G. (2013). Importance of Global Surveillance for Respiratory Syncytial Virus. J. Infec. Dise., 8 (1): S165-S166.

John, P. D. (2004). Natural Infection of Infants with Respiratory Syncytial Virus Subgroups A and B: A Study of Frequency, Disease Severity, and Viral Load. Pediatric Research. 56: 914–917.

Hussain, A. H. (2013). Detection of respiratory cyncytial virus and human metapneumovirus in hospital AL Ized children with respiratory tract infections. Thesis submitted to the council of the school of medicine at Sulaimani University, Iraq.

Shobugawa, Y.; Saito, R.; Sano, Y.; Zaraket, H.; Suzuki, Y.; Kumaki, A.; Dapat, I.; Oguma, T.; Yamaguchi, M. and Suzuki, H. (2009). Emerging genotypes of human respiratory syncytial virus subgroup A among patients in Japan. J. Clin. Microbiol., 47: 2475–2482.

Tapia, L.I.; Shaw, C.A.; Aideyan, L.O.; Jewell, A.M. (2014). Dawson, B.C.; Taha, R.H. and Pedro, A.P. Gene Sequence Variability of the Three Surface Proteins of Human Respiratory Syncytial Virus (HRSV) in Texas. PLoS ONE., 9(3): e90786.

Tabatabai, J.; Prifert, C.; Pfeil, J.; Grulich-Henn, J. and Schnitzler, P. (2014). Novel Respiratory Syncytial Virus (RSV) Genotype ON1 Predominates in Germany during Winter Season 2012–13. PLoS ONE 9(10): e109191.

Peter, L. C. and Graham, B.S. (2008). Viral and host factors in human respiratory syncytial virus pathogenesis. J. Virol., 82: 2040–2055.

Galiano, M. C.; Palomo, C.; Videla, C. M.;Arbiza, J.;Melero, J. A. and Carballal, G. (2005). Genetic and antigenic variability of human respiratory syncytial virus (groups A and B) isolated over seven consecutive seasons in Argentina (1995 to 2001). J. Clin. Microbiol. 43**:**2266-2273.

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Hsin, Chi.; Hsin-Fu, Liu.; Li-Chuan, Weng.; Nai-Yu, Wang.; Nan-Chang, Chiu.; Mei-Ju, Lai.; Yung-Cheng, Lin.;Yu-Ying, Chiu.;Wen-Shyang, H. and Li-Min, H.(2013). Molecular Epidemiology and Phylodynamics of the Human Respiratory Syncytial Virus Fusion Protein in Northern Taiwan. PLoS ONE., 8(5): e64012.

Fernanda, P.; Beck, C.; Souza, L.N.; Beatriz, Al.; Pinheiro, M.M.; Minuto, P.R.; Silva, M.D.; Pires, M.R.; Santos, R.P.; Kuchenbecker, R.S. and Barth, A.L. (2014). Evaluation of respiratory syncytial virus group A and B genotypes among nosocomial and community-acquired pediatric infections in southern Brazil Virol. J., 10:11-36.

Papenburg, J.; Carbonneau, J.; Marie-Ève, H.; Isabel, Sandra. ; Bouhy, X.; Ohoumanne, N .; Pierre, D.; Bosco, A. P.; Corbeil, J.; Michel, G.; Bergeron, G. De- S. and Boivin, G. (2012). Molecular Evolution of Respiratory Syncytial Virus Fusion Gene, Canada, 2006–2010.Emerging Infectious Diseases journal -CDC., 18(1).

Eshaghi, A.; Duvvuri, V.R.; Lai, R.; Nadarajah, J.T.; Li ,A.; Samir, N. Patel.; Donald, E. Low. and Jonathan, B. G. (2012). Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. PLoS ONE., 7 (3): e32807.

Pierangeli, A.; Trotta, D.; Scagnolari, C.; Ferreri, ML.; Nicolai, A.; Midulla, F.; Marinelli, K.; Antonelli, G. and Bagnarelli, P. (2014). Rapid spread of the novel respiratory syncytial virus A ON1 genotype, central Italy, 2011 to 2013. Euro Surveill., 19: (26):2.

Prifert, C.; Streng, A.; Krempl, C.D.; Liese, J. and Weissbrich, B. (2013). Novel respiratory syncytial virus a genotype, Germany, 2011–2012. Emerging Infect Dis., 19: 1029–1030.

Khor,C.S.;Ching Sam, I.; Poh-Sim, H. and Yoke-Fun, C.(2013). Displacement of predominant respiratory syncytial virus genotypes in Malaysia between 1989 and 2011.Infection, Genetics and Evolution., 14:357–360.

Table 1. Prevalence rate of RSV genotypes A and B in infant and young children

Age	No. of specimens	No. of RSV genotype A	No. of RSV genotype B	Rate of infection with RSV A
>2months to 1 year	37	11	non	11%
>1year to 2 years	35	11	non	11%
>2 years to 5 years	28	9	non	9%
Total	100	31	0	31%

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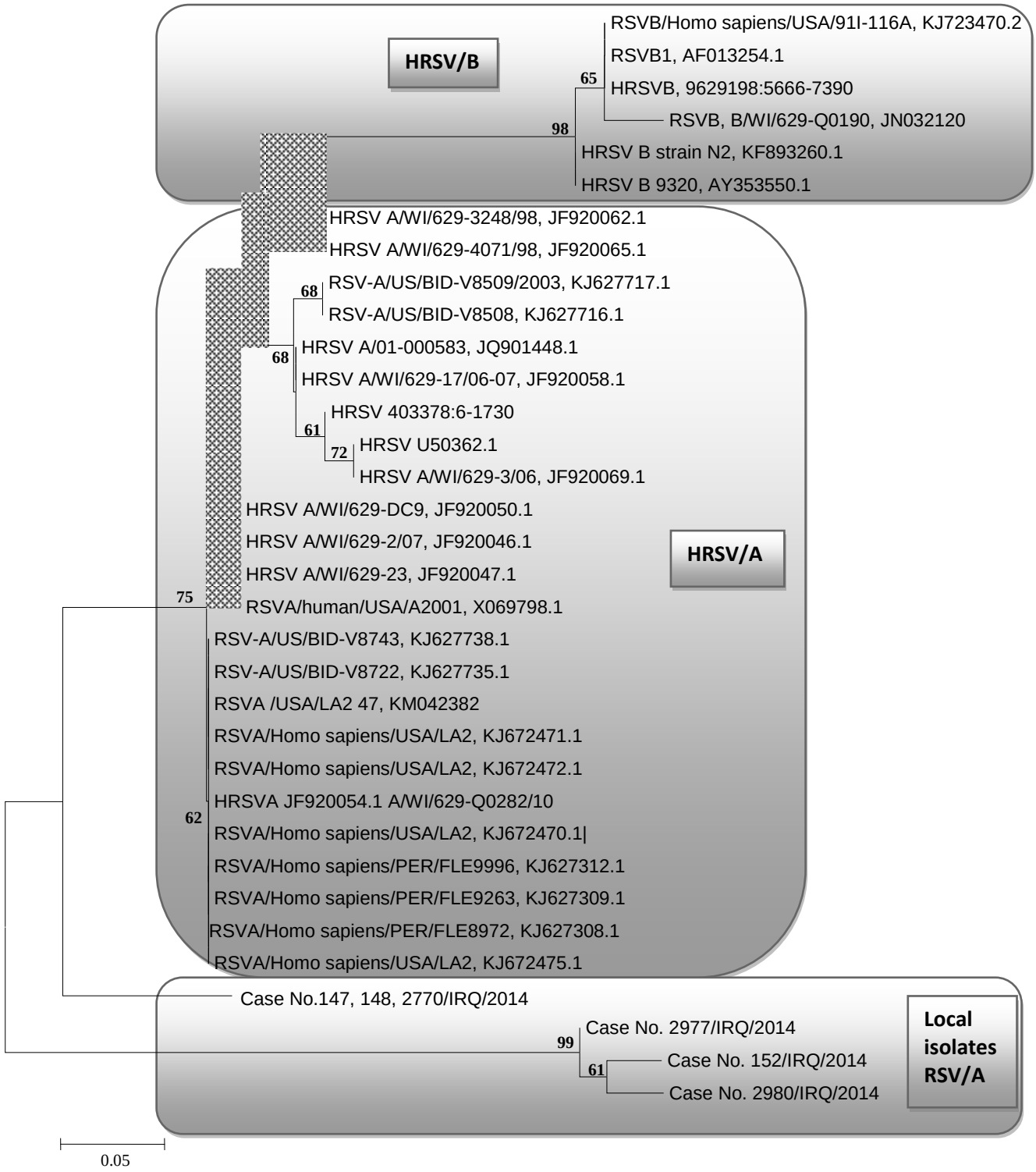

Figure 1. Maximum likelihood phylogenetic tree of partial F gene sequence (90 NTs) of RSV genotype A. Bootstrap replication of 500 times was conducted. Bootstrap value ≥ 60 was not shown in the tree (15). Sequences outputs of the local viral strain were compared with other 50 RSV A, RSV B virus. The local isolates clustered separately, though closer to the RSV A lineage. Evolutionary analyses were conducted using MEGA6.

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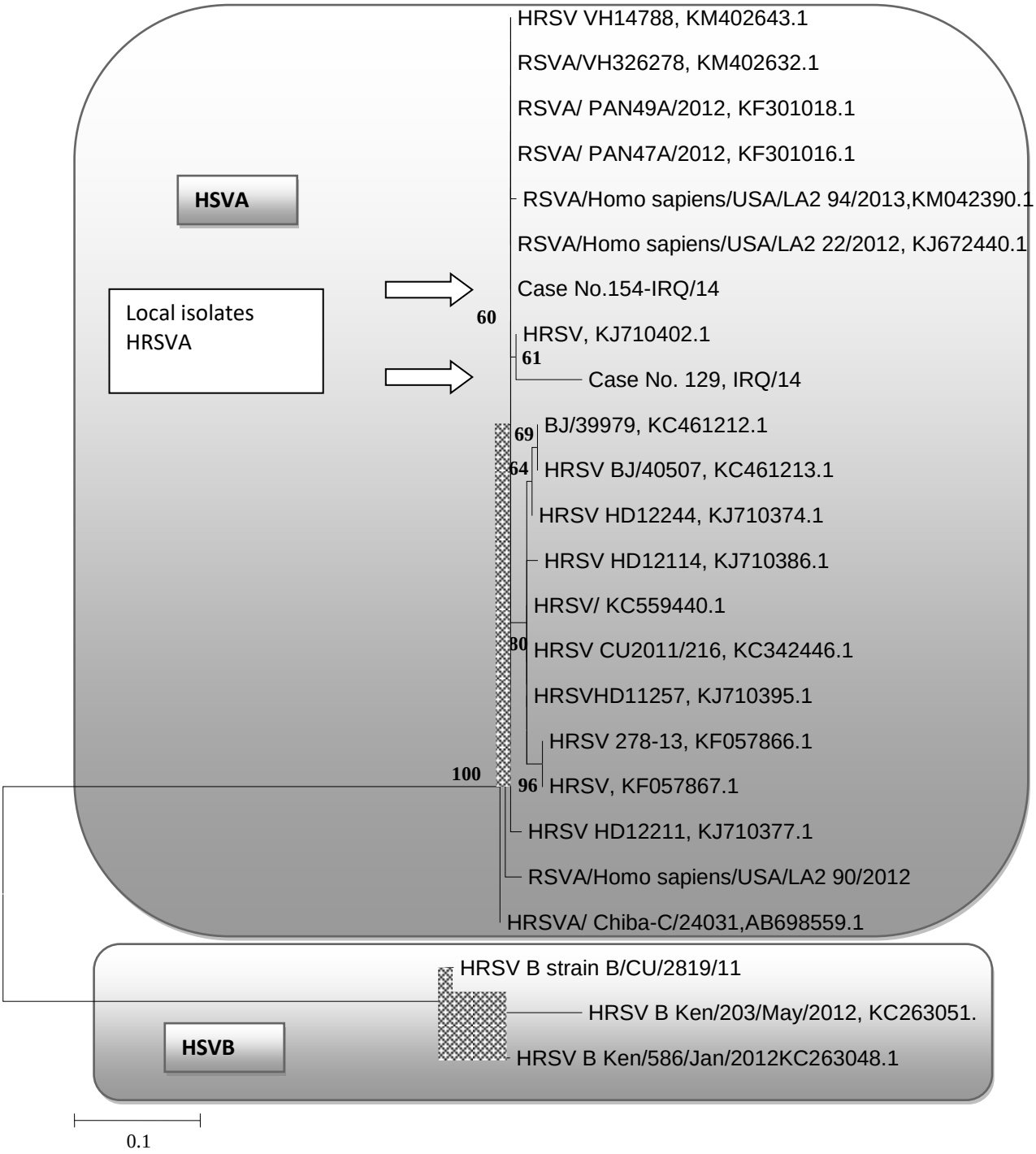

Figure 2. Phylogenetic analysis for partial sequence of G gene of RSV A local viral strains by Maximum Likelihood method. Bootstrap replication of 500 times was conducted. Bootstrap value ≥ 60 was not shown in the tree (16). The RSV A local isolates are clustered with a number of RSV A and RSV B viral strains. The Local RSV A isolate 129 is segregated with GenBank accession *KJ710402.1*. Isolates no. 154 was clustered with GenBank accession *KJ672440.1*. Evolutionary analyses were conducted using MEGA6.

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