Phylogenetic Analysis of Respiratory Syncytial Virus Isolated from Children with Respiratory Tract Infections in Baghdad City, Iraq

H L Abduljabbar¹, A A Hussein¹*, Q S Al-Mayah² and I M Auﬁ³

¹Department of Microbiology - College of Medicine - University of Diyala - Iraq.
²Medical Research Unit - College of Medicine - Al-Nahrain University - Iraq.
³National Influenza Center - Central Public Health Laboratory - Iraq.
*E-mail: Areej.2002@yahoo.com

Abstract: Respiratory syncytial virus (RSV) is the leading cause of hospitalization in infants worldwide, genotypes responsible of disease severity and host immune responses. This study aims to determine the infection rate of respiratory syncytial virus in children with respiratory tract infection and identify the genotyping among the study population. Cross sectional study which enrolled 150 infants with acute respiratory tract infection, males 81(54%) and females 69(46%) aged under five years old, who was admitted to Al-Imamin Al-Kadhimin Medical City and Pediatrics Protection Hospital in Baghdad during the period from December 2017 till April 2018. Nasopharyngeal swabs were collected from each participant and stored as frozen at -70 °C until to use for RNA extraction and convention polymerase chain reaction to detect of respiratory syncytial virus in the study population. According to result of this study out of all these samples, 26 samples were positive for RSV(17.33%). The infection rate of RSV is more common in males 17(65.39%), than females 9(34.61%) and in children ≤ one year (46.15%) also high frequency were noticed among patients live in an urban area (73.08%) and winter 20 (76.92%) than spring 6 (23.08%). According to different clinical feature, fever, cough, and wheezing were more common than other sign. The sequence conducted for all RSV- positive isolates, 11 respiratory syncytial virus positive isolates was in genotype B and 1 in genotype A. The sequence of RSV B the local isolates were closed to Argentina isolation and Tailwind isolate while in genotype A isolates were closed to isolates from different regions (Saudi Arabia, German, India isolation). The conclusion of this study revealed that respiratory syncytial virus B infections were more frequent than RSV A among children with acute respiratory tract infection.

1. Introduction

Human respiratory syncytial virus (RSV) is one of the most common viruses to infect children worldwide and increasingly is recognized as an important pathogen in adults, especially the elderly and immunocompromised patients [1]. Respiratory syncytial virus is responsible for more than three million yearly hospitalizations and up to 118 000 deaths in children under 5 years, is the leading pulmonary cause of death for this age group that lacks a licensed vaccine [2].

Respiratory syncytial virus particles are pleomorphic with both spherical and filamentous particles of different sizes; which comprise of a nucleocapsid bundled in a lipid envelope got from the host cell plasma membrane [3]. It is a cytoplasmic with linear, negative sense, ssRNA genome of approximately 15,000 nucleotides that is classified in the Pneumovirus genus of the Paramyxoviridae family. The viral genome encodes 11 proteins. Of these, the G- and F- proteins are the major surface antigens of RSV, which is involved in virus attachment to cell receptors and the mediation of cell membrane fusion, respectively. Both G- and F-proteins are accessible to neutralizing antibodies, however, only the G-protein is known to accumulate mutations in response to host immunological pressures [4].

Respiratory syncytial virus has been classified into groups A and B based on antigenic differences of the G, F, and N proteins [5]. Further genetic analysis of the nucleotide sequence of the second hypervariable region of the G gene allowed for the classification into
genotypes within antigenic groups RSV - A and B [6]. Many studies have reported the molecular epidemiology and genetic variability of RSV worldwide [7, 8, 7]. Group A strains have been categorized into 14 genotypes (GA1 to GA7, SAA1, CB-A, NA1 to NA4 and ON1) [8]. While subgroup B is categorized into 27 genotypes (BA1 to BA12, BA-C, SAB1 to SAB4, GB1 to GB4, URU1 to URU2, CB-B, CB-1, BA-CCB and BA-CCA) [9].

Respiratory syncytial virus is transmitted through close contact with a person who has an active infection or direct contact with infectious secretions on environmental surfaces such as droplets, saliva, or large particle aerosols. Although fomites may also be a source of contamination [10].

Signs and symptoms of respiratory syncytial virus infection most commonly appear about four to six days after exposure to the virus. In children, RSV usually causes mild cold-like signs and symptoms. These include fever, congested or runny nose, sore throat, dry cough and mild headache [11]. And then progress down into the lower respiratory tract to cause bronchiolitis, pneumonia, and they implicated with allergy and asthma exacerbation [12].

The present study aims to determine the infection rate and genotyping of human respiratory syncytial virus in children with acute respiratory tract infection.

2. Patients and Methods

A cross sectional study was based on the processing of nasopharyngeal swab from 150 children with acute respiratory tract infections, aged under five years old, who was admitted to Al-Imamin Al-Kadhimin Medical City and Pediatrics Protection Hospital in Baghdad during the period from December 2017 till April 2018. Data were collected by interview with a parent or relevant of each participant, through structural questionnaire which include gender, age, residence, season, type of infection, cough, fever, wheezing, nasal discharge, history of asthma, diarrhea and nervous manifestations.

Swab specimens were preserved in viral transport media (VTM) without antibiotics, according to [15]. Then transported by a cool box to the Virology Unit at the National Central Public Health Laboratory. All samples were vortexes for 15 seconds to dislodge material on the swab into the transport medium. On completion of routine investigations of microbial causes of respiratory tract infection all residual nasopharyngeal aspirate samples were divided into aliquots, labelled and stored at -70°C until the time of analysis.

RNA extraction

The viral RNA to be used as a template for cDNA synthesis was extracted directly from nasopharyngeal swabs by using QIAamp Viral RNA Mini Kit (Cat. No. 52904, 52906, QIAGEN, Germany).

PCR technique

For the external PCR, GPA (nt511-530, 5'-GAAGTGTTCAACTTTGTACC-3') for subgroup A and GPB (nt 494-515, 5'-AAGATGATTACCATTTTG AAGT-3') for subgroup B were used as forward primers. Hemi-nested PCR was carried out with subgroup A-specific forward primer, nRSAG (nt 539-558, 5'- TATGCAGCAACAATCCAACC-3'), and subgroup B-specific forward primer, nRSBG (nt 512-531, 5'-GTGGCAACAATCAACTCTGC-3'). In the external and hemi-nested PCR, primer F1 (nt
3'-CAACTCCATTGTTATTGCC-3') was used as reverse primer for both subgroups A and B[16].

In the nested reverse transcription polymerase added 8μl of template RNA to the component of One Step RT-PCR Kit (Cat.No.210210, 210212, and 210215, QIAGEN, Germany), consisting of 4 μl 5X buffer, 0.8 μl dNTPs, 0.8 μl Enzyme Mix, 1 μl of Mgc2,1.2 μl of Forward and Reverse primers and 3 μl of RNase-free water. The total reaction volume was 20 μl. Protocol that used in a nested reverse transcription - polymerase chain reaction assay to amplify a fragment of the RSV G gene to detect of subgroup A and subgroup B respectively consisted of 30 min at 50°C of reverse transcription, Initial PCR activation at 15 min for 94°C, 35 cycle of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min and a final extension at 72°C for 7 min, two μl of external PCR product was used for semi-nested PCR amplify a fragment of the RSV G gene was used to detect of subgroup A and subgroup B. Added to the component of Master Mix: Go Taq® green master mix (Cat No.K-2018 Promega -USA) consisting of 12.5μl Green master mix, 12.5 μl of Forward and Reverse primers and 8μl of Nuclease free-water. The total reaction volume was 25 μl.

A protocol that used in semi-nested PCR assay to amplify a fragment of the RSV G gene was used to detect of subgroup A and subgroup B consisted of 94°C for 30 min, 30 cycles of 94 for 30 Sec, 50 for 45 Sec and 72 for one min and a final extension at 72°C for five min.

The external and hemi-nested PCR amplicons were 550 and 500 bp, respectively. Both subgroups A and B.

**Sequencing of PCR products and data analysis.**

After successful amplification of the target regions of RSV by using primers, (25μl) of PCR product along with primers, were sent abroad to Macrogen company in South Korea for direct sequencing. Homology search was conducted using basic local alignment search tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) to identify the resultant sequencing, then all local isolate were recorded in Gene Bunk

**3. Results**

The rate of respiratory syncytial virus infection among children under five years age was 17.33 % (26 out of 150) samples using conventional-PCR. Group A was 30.78% and group B was 69.23% as shown in (Figure 1 and 2).

![Figure (1): Gel electrophoresis of the second round PCR amplification for G gene (550bp). Line M represents DNA ladder (100bp), line1, 2, 3, 4, 5, 6, 7, 8 represent PCR product of RSV group A, stained with ethidium bromide and illustrated under UV light.](attachment:image.png)
Figure (2): Gel electrophoresis of the second round PCR amplification for G gene (500bp). Line M represents DNA ladder (100bp), line 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 represent PCR product of RSV group B, stained with ethidium bromide and illustrated under UV light.

Respiratory syncytial virus infection is predominant in males 17 (65.38%) than female 9 (34.62%), age group 1-12 month (46.15%) than another 13-24 months (46.15%), 25-36 months (19.23%) and 37-48 months (7.69%). The sequence conducted for all RSV- positive isolates, 11 respiratory syncytial virus positive isolates was 10 in subgroup B and 1 in subgroup A. The sequence of the local isolates RSV subgroup B were closed to Argentina isolation and Tailwind isolation while in RSV subgroup A isolates were closed to isolate from different regions (Saudi Arabia, German, India, China isolation. According to phylogenetic tree Figure (3).

Figure (3): Phylogenetic tree for protein G genes (RSV) constructed by the neighbor joining method for 11 local isolates from nasopharyngeal swabs and 14 reference isolate from Gene Bank. Phylogenetic distances were measured by the kimura two-parameter, model of the tree was statistically supported by bootstrapping with 1000 replicates. Bootstrap values below 50% are not shown. Current isolates are indicated with red circles.
4. Discussion

According to the results of the current study, the infection rate of RSV was 17.33% using conventional PCR. This result is comparable with several studies conducted in different areas such as study done by Al-Charrakh et al., (2016) recorded (18.75%) in asthmatic patients used real time polymerase chain reaction in Wasit city [17], and with Hassan et al., (2018) revealed a seroconversion rate of RSV was (20.4%) among young children in the Kurdistan region [18].

The infection rate of RSV in this study is relatively low compared with data reported in Baghdad by Odisho et al., (2009) the percentage is reached to 79% among the children who have respiratory tract infection [19]. In neighboring countries the infection of the RSV was (36.8%) by Khadadah et al., (2010) in hospitalized children with respiratory tract infection in Kuwait [20] and to study done by Parsania et al., (2016) shows that 31.1% of RSV infections among Iranian children < 5 years of age [21]. In this study the infection rate seems to be higher with than other studies such as study done by Atyah et al., 2017 who recorded (6.6%) in children under 15 years old in Baghdad [22].

These variations in incidence among studies might reflect different epidemiological patterns of RSV infection in different countries, which in turn might be related to environmental factors, geographical factors, differences in host genetic susceptibility, immune status, sampling size, detection techniques, and different viral strains circulating in different geographical areas [23].

According to gender, it has been found that RSV infection in males more than females seems to be similar with those who participated in other studies such as Rodriguez-Fernandez et al., (2017) in Texas [24], Zahran et al., (2017) in Egypt [25], Hassan et al., (2018) in Iraq[18]. And Jepsen et al., (2018) Infant boys were at higher risk of severe RSV infection as compared to infant girls in Denmark [26]. While the current study is inconsistent with a study conducted by Reina et al., (2008) which revealed that the gender, females (53.2%) was higher than males [27].

Regarding the age, it has been found that RSV infections were higher among age group <1 year, 12(46.15%) use conventional-PCR technique, when compared to older children. This result was comparable to that Chen et al.,(2010) in china [28], Khalil et al., (2015) in Egypt [29], Al-Mossawi et al., (2016) in Iraq; the incidence of severe RSV infections was highest among infants and young toddlers and peaked in the 1 to 2 month-old infants [30].

Result of phylogenic analysis, which demonstrated that all local isolated did not had any similarity with references isolate at gene bank, this could be related with limited studies done around Iraq as well as this is the first study done in Iraq to determine the genotyping according to our knowledge. This study found a remarkably higher rate of RSV subgroup B (69.23%) than RSV subgroup A (30.78%), this result is in agreement with a study done by an Al-Mossawi et al., (2016) who recorded 8% and 14% of respiratory syncytial virus type A and B respectively, among children suffering from respiratory tract in Al-Amarah city [30]. And with study done by a Kenmoe et al., (2018) found RSV group A and group B co-circulated in this population at 17.4 and 82.6%, respectively [31]. On the other hand, many studies report the same results conducted in different areas such as (Hirsh et al., 2014; Gimferrer et al., 2015; Liu et al., 2014) [32, 33, 34]. While this result was disagreement with other studies such as Fieldhouse et al., (2018) revealed a high frequency of RSV-A(30.2% overall) among patients with pneumonia admitted to the two hospitals in Sarawak, Malaysia [35]. Faghihloo et al., (2011) found 66.6% RSV-positive belonged to subgroup A while others to B (33.4%) [16]. Also, this result disagreed with other global studies such as [36, 24]. On the other hand large sample size study consists of 1589 hospitalized children with bronchiolitis from 2007 through 2010 in the U.S.A found no difference in disease severity between infants with RSV-A and RSV-B [37]. Another study found two types of RSV responsible for all seasonal outbreaks [38]. Regarding severity Some studies have reported that RSV-A demonstrated a higher (more severe) clinical score index in RSV-A infection [39], on the basis of respiratory rate, wheezing, heart rate, difficulty in feeding and oxygen saturation associated with more severe clinical disease [40]. While in few other studies group B infections have
been reported to cause more severe disease [41]. This difference could be related to sample size and seasons. Infectivity of the virus, the development of immunological resistance in the community, and viral genetic drift due to spontaneous mutation may be important in the patterns of seasonal circulation and genetic evaluation of RSV strains [42].

The sequence conducted for all RSV-positive isolates, 10 respiratory syncytial virus positive isolates was in genotype B and I in genotype A. The sequence of RSV B the local isolates were closed to Argentina isolation and Tailwind isolation while in genotype A isolates were closed to isolate from different regions (Saudi Arabia, Germany, India isolation). This could be related to increase travel in a different area for tourism, treatment, study as well as increase in the religious travel. This contradiction could be attributed to difference in study design and population, definition of disease severity, the distribution of RSV subtypes [32]. And the interplay between host and virus factors, including RSV load [43]. Also a possible explanation for these alterations is the development of specific immunity against a specific RSV type that is prevalent in the country in the preceding year. For that, subgroups shift of RSV from year to year that may affect the immunity acquired against the previously circulating viruses [44].

5. Conclusions
The RSV subgroup B was more than subtype A by conventional PCR. All local RSV subgroup B strains were closed to Argentina isolation and Tailwind isolation while in genotype A isolates were closed to isolate from different regions (Saudi Arabia, Germany, India, China isolation). Further studies with large sample size to clarify this issue and identify new local isolates.

6. Acknowledgements
The authors would like to thank all participant in this study, as well as the director and staff of Virology Unit at the National Central Public Health Laboratory at Baghdad city, for their support in samples processing.

7. References
[1] Domachowske J, Halczy J and Bonville CA 2018 Preventing pediatric respiratory syncytial virus infection. *Pediatr. Ann.* **49**(9):e371-e376.
[2] Caballero MT, Polack FP 2018 Respiratory syncytial virus is an “opportunistic” killer. *Pediatric pulmonology* **53**(5):664-7.
[3] Collins PL, Fears N and Graham BS 2013 Respiratory syncytial virus: virology, reverse genetics, and pathogenesis of disease. In challenges and opportunities for respiratory syncytial virus vaccines, Springer. Berlin. Heidelberg. pp. 3-38.
[4] King AM, Adams MI, Carstens EB and Lefkowitz EJ 2012 RSV Collaborative Study Group. Molecular epidemiology of human respiratory syncytial virus among children in Japan during three seasons and hospitalization risk of genotype ON1. *PloS One* **13**(1):e0192085.
[5] Ahmed A, Haider SH, Parveen S, Arshad M, Alsenaidy HA, Baaboud AO, Mobaireek KF, AlSaadi MM, Alsenaidy AM and Sullender W 2016 Co-Circulation of 72bp duplication group A and 60bp duplication group B respiratory syncytial virus (RSV) strains in Riyadh, Saudi Arabia during 2014. *PloS One* **11**(11):e0166145.
[6] Ahmed A, Haider SH, Parveen S, Arshad M, Alsenaidy HA, Baaboud AO, Mobaireek KF, AlSaadi MM, Alsenaidy AM and Sullender W 2016 Co-Circulation of 72bp duplication group A and 60bp duplication group B respiratory syncytial virus (RSV) strains in Riyadh, Saudi Arabia during 2014. *PloS One* **11**(11):e0166145.
[7] Ahmed A, Haider SH, Parveen S, Arshad M, Alsenaidy HA, Baaboud AO, Mobaireek KF, AlSaadi MM, Alsenaidy AM and Sullender W 2016 Co-Circulation of 72bp duplication group A and 60bp duplication group B respiratory syncytial virus (RSV) strains in Riyadh, Saudi Arabia during 2014. *PloS One* **11**(11):e0166145.
[9] Bashir U, Nisar N, Mahmood N, Alam MM, Sadia H, Zaidi SSZ 2017 Molecular detection and characterization of respiratory syncytial virus B genotypes circulating in Pakistani children. *Infect. Gen. Evol.* 47:125-131.

[10] Ren L, Xia Q, Xiao Q, Zhou L, Zang N, Long X, Xie X, Deng Y, Wang L, Fu Z and Tian D 2014 The genetic variability of glycoproteins among respiratory syncytial virus subtype A in China between 2009 and 2013. *Infect. Genet. Evol.* 27:339-47.

[11] Zhang L, Liu W, Liu D, Chen D, Tan W, Qiu S, Xu D, Li X, Liu T and Zhou R 2018 Epidemiological and clinical features of human metapneumovirus in hospitalised paediatric patients with acute respiratory illness: a cross-sectional study in Southern China, from 2013 to 2016. *BMJ open* 8(2):e019308.

[12] Haas L, Thijsen S, Van Elden L and Heemstra K 2013 Human metapneumovirus in adults. *Viruses* 5(1):87-110.

[13] Meissner HC 2016 Viral bronchiolitis in children. *N. Engl. J. Med.* 374(1):62-72.

[14] Rezaee F, Linfield DT, Harford TJ and Piedimonte G 2017 Ongoing developments in RSV prophylaxis: a clinician’s analysis. *Curr. Opin. Virol.* 24:70-8.

[15] Moe N, Krokstad S, Stenseng IH, Christensen A, Skanke LH, Risnes KR, Nordbø SA and Dollner H 2017 Comparing human metapneumovirus and respiratory syncytial virus: viral co-detections, genotypes and risk factors for severe disease. *PloS One* 12(1):e0170200.

[16] Faghihloo E, Salimi V, Rezaei F, Naseri M, Mamishi S, Mahmoody M and Mokhtari Azad T 2011 Genetic diversity of the G protein gene of human respiratory syncytial virus among Iranian children with acute respiratory symptoms. *Iranian J. Pediatr.* 21(1):58.

[17] Al-Charrakh AH, Ghanim AA and Jalal ATA 2016 Detection of human metapneumovirus and respiratory Syncytial Virus associated with asthmatic patients using direct fluorescent assay and Real time - PCR. *Wasit J Sci Med* 8(4): 52-65.

[18] Hassan DA, Rachid SK and Ziebuhr J 2018 A Single-Center Study of Viral Respiratory Tract Infections in Hospitalized Children From the Kurdistan Region of Iraq. *Glob. Pediatr. Health* 5:1-8.

[19] Odisho SM, Al-Bana AS and Yaassen NY 2009 Detection of Respiratory syncytial virus infection in a sample of infants in Iraq. *Iraqi J. Med. Sci.* 7(4): 11-19.

[20] Khadadah M, Essa S, Higazi Z, Behbehani N and Al-Nakib W 2010 Respiratory syncytial virus and human rhinoviruses are the major causes of severe lower respiratory tract infections in Kuwait. *J. Med. Virol.* 82(8):1462-7.

[21] Parsania M, Behzad P, Mohammad HP, Sama H, Aref A and Alireza N 2016 Detection of human metapneumovirus and respiratory syncytial virus by real-time polymerase chain reaction among hospitalized young children in Iran. *Jundishapur J. Microbiol.* 9(3): e32974.

[22] Ayat N, Hula YF, Faisal GA, Iman MA and Manal AA 2017 Molecular detection of subfamily pneumovirinae among children with flu-like illness by using RT-PCR. *Curr Res. Microbiol. Biotechnol.* 5(5): 1239-1244.

[23] Panayiotou C, Richter J, Koliou M, Kalogiriou N, Georgiou E and Christodoulou C 2014 Epidemiology of respiratory syncytial virus in children in Cyprus during three consecutive winter seasons (2010–2013): age distribution, seasonality and association between prevalent genotypes and disease severity. *Epidemiol. Infect.* 142(11):2406-11.

[24] Rodriguez-Fernandez R, Tapia LI, Yang C, Torres JP, Susana Chavez-Bueno S, Garcia C, Jaramillo LM, Moore-Clingenpeel MS, Jafari HS, Peebles ME, Piedra PA, Ramilo O and Mejias A 2017 Respiratory Syncytial Virus Genotypes, Host Immune Profiles, and Disease Severity in Young Children Hospitalized With Bronchiolitis. *J. Infect. Dis.* 217: 24-34.

[25] Zahran WA, Makled AF, Salama AA, El-Hendaw GR and Bader HS 2017 Comparison of reverse transcription-PCR and viral culture for detection of respiratory syncytial virus in young children: relation to epidemiological and clinical findings. *Egypt J. Med. Microbiol.* 26(2):27-36.

[26] Jepsen MT, Trebbien R, Emborg HD, Krause TG, Schønning K, Voldstedlund M, Nielsen J and Fischer TK 2018 Incidence and seasonality of respiratory syncytial virus hospitalisations in young children in Denmark, 2010 to 2015. Euro surveillance: bulletin European sur les maladies transmissibles = European Communicable Disease Bulletin 23(3):

[27] Reina J, Ferrés F, Mena A, Figuerola J and Alcoceba E 2008 Clinical and epidemiological characteristics of respiratory infections caused by human metapneumovirus in pediatric patients. *Enferm. Infeccc. Microbial. Clinica* 26(2):72-6.
[28] Chen X, Zhang ZY, Zhao Y, Liu EM and Zhao XD 2010 Acute lower respiratory tract infections by human metapneumovirus in children in Southwest China: A 2-year study. Pediatr. Pulmo. 45(8):824-31.
[29] Khalil SO, Enan KA, Ali YH, Salim B, Watt IME and Zardis M 2015 Detection and Molecular Characterization of Respiratory Syncytial Virus (RSV) in children with respiratory signs in Khartoum state, Sudan 2011-2012. Am. J. Infect. Dis. Microbiol. 3(1): 6-13.
[30] Al-Mossawi, MMK, Al-Hamadani AA and Al-Hilali, AH 2016 Molecular Investigation Of Human metapneumovirus and Respiratory syncytial virus in Children in Al-Amarah City, Ph.D. Thesis, College of Medicine - University of Al-Qadisiayah, Iraq.
[31] Kenmoe S, Vernet MA, Miszczak F, Dina J, Schoenhals M, Beng VP, Vabret A and Njouom R 2018 Genetic diversity of human respiratory syncytial virus isolated among children with acute respiratory infections in Southern Cameroon during three consecutive epidemic seasons, 2011-2013. Trop. Med. Health 46(1):7.
[32] Hirsh S, Hindiyeh M, Kolet L, Regev L, Sherbany H, Yaary K, Mendelson E and Mandelboim M 2014 Epidemiological changes of respiratory syncytial virus (RSV) infections in Israel. PloS One 9(3):e90515.
[33] Gimferrer L, Campins M, Codina MG, del Carmen Martín M, Fuentes F, Espejarla J, Bruguera A, Vilca LM, Armadans L, Pumarola T and Antón A 2015 Molecular epidemiology and molecular characterization of respiratory syncytial viruses at a tertiary care university hospital in Catalonia (Spain) during the 2013-2014 season. J. Clin. Virol. 66:27-32.
[34] Liu J, Mu Y, Dong W, Yao F, Wang L, Yan H, Lan K and Zhang C 2014 Genetic variation of human respiratory syncytial virus among children with fever and respiratory symptoms in Shanghai, China, from 2009 to 2012. Infect. Genet. Evol. 27:131-6.
[35] Fieldhouse JK, Teck-Hock T, Wei-Honn L, Jackie T, Siaw-Jing H, King-Ching H, Cheng-Ink G, Toh-Mee W, See-Chang W, Tyler EW and Gregor, CG 2018 Surveillance for respiratory syncytial virus and parainfluenza virus among patients hospitalized with pneumonia in Sarawak, Malaysia. PloS One 13(8): E0202147.
[36] Meng J, Stobart CC, Hotard AL, Moore ML 2014 An overview of respiratory syncytial virus. PloS Pathogens 10(4):e1004016.
[37] Laham FR, Mansbach JM, Piedra PA, Hasegawa K, Sullivan AF, Espinola JA and Camargo CA 2017 Clinical profiles of respiratory syncytial virus subtypes A and B among children hospitalized with bronchiolitis. The Pediatric Infectious Disease Journal 36(8):808-10.
[38] Rebuffo-Scheer C, Bose M, He J, Khaja S, Ulatowski M, Beck ET, Fan J, Kumar S, Nelson MJ and Henrickson KJ 2011 Whole genome sequencing and evolutionary analysis of human respiratory syncytial virus A and B from Milwaukee, WI 1998-2010. PloS One 6(10):e25468.
[39] Papadopoulos NG, Gourgiotis D, Javadyan A, Bossios A, Kallergi K, Psarras S, Tsolia MN and Kafetzis D 2004 Does respiratory syncytial virus subtype influences the severity of acute bronchiolitis in hospitalized infants. Respiratory Medicine 98(9):879-82.
[40] Jafri HS, Wu X, Makari D and Henrickson KJ 2013 Distribution of respiratory syncytial virus subtypes A and B among infants presenting to the emergency department with lower respiratory tract infection or apnea. Pediatr Infect Dis J 32(4):335-40.
[41] Tran DN, Pham TM, Ha MT, Tran TT, Dang TK, Yoshida LM, 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.
[42] Sullender WM 2000 Respiratory syncytial virus and antigenic diversity. Clin. Microbial. Rev. 13(1): 11-15.
[43] Fodha I, Vabret A, Ghedira L, Seboui H, Chouchane S, Dewar J, Gueddiche N, Trabelsi A, Boujaafar N and Freymuth F 2007 Respiratory syncytial virus infections in hospitalized infants: association between viral load, virus subgroup, and disease severity. J. Med. Virol. 79(12):1951-8.
[44] Iwane MK, Farnon EC and Gerber SI 2013 Importance of global surveillance for respiratory syncytial virus. Pediatr Infect Dis J 208(Suppl-3):S165-6.