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Molecular detection of Subfamily Pneumovirinae among Children with Flu-like illness by using RT-PCR

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ABSTRACT
Acute respiratory tract infections (ARTI) are responsible for considerable morbidity and mortality in humans; especially among children, elders and immune-compromised individuals around the world. This study aimed to focus on the prevalent human metapneumovirus (hMPV) and respiratory syncytial virus (RSV) of the upper and lower respiratory tract that causes severe and acute infections among children below the age of 15 years in Iraqi community. A total 195 nasopharyngeal swabs samples were collected from patients having flu-like illnesses; during time between September 2016 and May 2017. All samples were tested under molecular criteria for both viruses by using "Real time PCR" techniques. Result showed 58 (29.74%) positive for hMPV, and 13 (6.67%) other samples were positive for RSV. The mean age of hMPV and RSV was 33.28±5.3 and 19.93±4.8 month, respectively and more than 60% of infected cases were under 3 years' old children. This represented the highest significant difference of hMPV infection in young children of other age groups. Although the RSV cases observed in children less than 2 years, there is no significant difference among infected children. On the other hand, the frequency of hMPVs among the months showed high significant fluctuation likely in Oct, Nov and Jan months. But nearly spring and late winter months, especially Feb and Mar showed the highest percentage of the RSVs infection (seasonal infection) with slightly statistical significant differences between them. In conclusion, performing the multiplex RT-PCR assay to report the molecular epidemiology of Paramyxoviridae in respiratory tract along with influenza virus during flu season.

Keywords: human Metapneumovirus, Respiratory syncytial virus, ILI, RT-PCR, ARTI.

1. INTRODUCTION
Respiratory infections are the most common illnesses among children throughout the world [1]. The viral etiologies are primarily associated with most of the viruses belonging to the families of Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Adenoviridae, and Coronaviridae [2].

The family of Paramyxoviridiae has a non-segmented, single stranded, negative-sense 15Kb RNA genome [3]. This family is classified into two subfamilies, the Paramyxovirinae and Pneumovirinae, the *Paramyxovirinae* subfamily includes two other genera: *Morbillivirus* (measles virus) and the *paramyxoviruses* (mumps virus and *parainfluenza* viruses) [4-6]. Meanwhile, other subfamily the "Pneumovirinae" consists of two genera: *Metapneumovirus* genus (human Metapneumovirus- hMPV) and the genus *Pneumovirus* (respiratory syncytial virus-RSV) [7,8].

The Human Metapneumovirus (hMPV) is closely related to the avian pneumovirus type C. Clinically; it resembles the respiratory syncytial virus (RSV), a common respiratory pathogen considered globally as a major cause of human morbidity and mortality , the spectrum of illnesses ranges from upper respiratory tract infections (URTI), lower respiratory tract

infections (LRTI) to severe bronchiolitis and pneumonia. These viruses are common in children less than 5 years of age, it also can be found in adults of all age groups [9, 10].

HMPV and RSV infections occur worldwide with a broad clinical spectrum from mild to severe and sometimes life threatening affection. HMPV infections can occur throughout the year, but seasonality has been described in several studies, with the epidemiological peak occurring 1 to 2 months later than that observed for RSV epidemics [11, 12].

HMPV infection is usually limited to the respiratory tract. The lower respiratory illnesses most frequently caused by HMPV are bronchiolitis, pneumonia, croup, and asthma exacerbation. Clinical signs and symptoms of HMPV infection overlap with those for other common respiratory viruses [13]. Reinfection with HMPV occurs, although repeated infections are more likely to be limited to the upper respiratory tract in otherwise healthy children [14, 15].

Respiratory syncytial virus (RSV) is a very contagious virus usually causes only a mild cold in healthy adults and elder children [16], it is one of the most common etiological agents of viral lower respiratory tract infection and is considered as the most important cause of viral bronchiolitis in children [17]. In developing countries, it has been found that RSV is detected in 6-96% of cases of hospitalized lower respiratory tract infections. A difference in RSV rates in developing countries also depends on many social factors, and the peak for RSV correlates with the presence or absence of rainfall [18]. Coinfection of hMPV with RSV in infants has been suggested to be a factor that influences the severity of bronchiolitis [19].

However, the detection difficulties in cell culture, and the viral slow growth and mild cytopathic effects (CPE) in cell culturing, this encourage the molecular technique was applied to diagnosis the respiratory viral agents like hMPV and RSV [20-22].

In Iraq, the Pneumovirinae viruses exist and available data few especially with increasing negative cases of influenza virus during the winter season. Hence, the current study aimed to detect RSV and hMPV presence as a Pneumovirinae subfamily in nasopharyngeal by real-time PCR from children of moderate-to-severe pneumonia with influenza-like illness.

2. MATERIALS AND METHODS

2.1 Samples

The study was conducted at the Iraqi Central Public Health Laboratory (CPHL), National Influenza Center (NIC), Baghdad/Iraq, during the time period from September 2016 to May 2017. A total of 195 nasopharyngeal swabs samples were included in the study; all samples were collected from children with age range of 1 month -15 years. All samples were selected negative for influenza A & B, patients were symptomatic to ILI" Influenza like Illnesses". All swabs were collected in clean, labeled, screw capped container that contains VTM" Viral transport media" for survival of virus alive until its arrival to laboratory and in accordance with routine procedures, followed by transportation of swabs by a cool box to the department of virology at the national central public health laboratory, samples stored undiluted at -80°C (deep freeze) until the time of analysis.

2.2 RNA extraction

All Nasopharyngeal swabs samples selected for molecular assay were first conducted for Viral RNA extraction, by using the QIAamp Viral RNA Mini kit (Qiagen GmbH, German), as described by the manufacturer's instructions, with few modifications on the process for optimal results. Frozen samples were first taken out of the deep freezing system, to be thawed at room temperature as the first step before initiating the extraction procedure. The kit procedures were conducted with few modifications to reach the optimal product yield.

2.3 Real-Time PCR (RT-PCR) Amplification

The process of Real time PCR for both viruses was conducted in a 7500 fast Applied Bio- systems instrument.

2.3.1 hMPV

One pair of specific primers are tested to reverse transcribe and amplify the human metapneumovirus highly conserved F genes. Primers were highly specific for the F capsid genes of the HMPV, the forward sequence primer was (5'-CAAGTGTGACATTGCTGAYCTRAA -'3), reverse primer (5'-ACTGCCGCACAACAACATTTAGRAA-'3) and Probe (5'-TGGCYGTYAGCTTCAGTCAATTCAACAGA -'3) [23]. The probe for hMPV uses FAM dye on the 5' reporter end and a non- fluorescent dye on the 3' quencher end .The master mix in use for the one step RT-PCR was the (QIAGEN) mix reagents. It was added to 10 µl RNA templates, 0.5 pmol conc. of each primer and 0.3 pmol conc. of the probe in 25 µl reaction mixture. Amplification and detection were done with an Applied Biosystem7500. Briefly, one cycle for 30 min at 50C° and 15min at 94C°, followed by 45 cycles for 15 s at 95C° and 1 min at 60C°.

2.3.2 RSV detection

For the RSV F gene detection, the amplification done by using forward primer sequence (5′AACAGATGTAAGCAGCTCCGTTATC-′3), with reverse primer (5′CGATTTTTATTGGATGCTGTACATTT-′3) and probe (5′TGCCATAGCATGACACAATGGCTCCT-′3). The probe was labeled with 5′ reporter dye FAM and the′3 quencher dye TAMRA [24]. The master mix in use for the one step RT-PCR was the (QIAGEN) mix reagents. (QIAGEN) were added to 10 µl RNA templates, 0.5 pmol conc. of each primer and 0.3 pmol conc. of the probe in 25 µl reaction mixture. Amplification and detection were done with an Applied Biosystem7500. Briefly, one

cycle for 30 min at 50C° and 15min at 94C°, followed by 45 cycles for 10 s at 95C° and 1 min at 60C° (7).

2.4 Statistical analysis

The statistical analysis system was analyzed by IBM SPSS Statistics 20 Brief Guide [25]. All values were calculated according to the positive results as percentages (%). Differences between study groups and assays were analyzed by cross-tab and Pearson chi-square (X^2) test. A value of P<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

RT-PCR has the ability to detect 1-1000 ng viral RNA and do not depend on intact viral particles [26], thus this assay is to confirm the identification of both hMPV & RSV infection in children under the age of 15 years. In general, this study used the specific primers and probe the F gene that were designed to detect hMPV & both subtypes of the RSV; types A and B. The F gene is the highest conserved with mostly synonymous changes leading to greater NT than AA diversity, as described by many authors [27-29].

Results of RT-PCR reported positive viral RNA for both viruses is 36.41% (71/195) of children with ILI symptoms, 29.74% (58/195) of those samples are hMPV positive, while 6.67%(13/195) are RSV positive. The remaining percentage 63.59% of the tested samples showed negative results (124/195) (Fig. 1). In previous local experiments for the detection of hMPV and RSV in RTI, they reported different values ranged 8-16% and 14-19%, respectively [30-33]. These differences might due to many factors such as technique, province, gene detection and period time of research.

In contrast, other parts of the world reported different values a high incidence of RSVs and fewer percentage of hMPVs in our study [34-38].

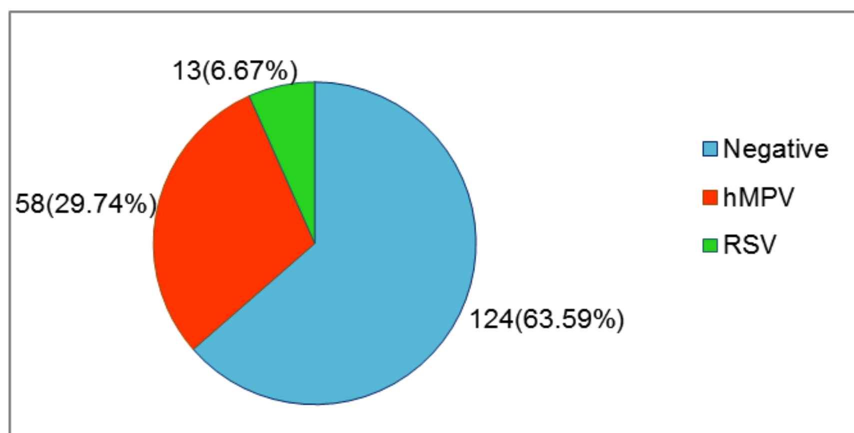

Figure 1: Distribution of Pneumovirinae subfamily according to RT-PCR assay in infected children with FLI

The statistical analysis gave a significant difference between viral infections of pneumovirinae and the negative cases (X^2= 14.405; P<0.01), where the viral RNA was detected in approximately a third (36.41%) of the total study groups. However an increase in cases of hMPV and other respiratory tract viruses might be due to increased use of molecular techniques for diagnosis like real time PCR.

Meanwhile, the negative cases of suspected respiratory tract infection for subfamily Pneumovirinae may explain other causes of RTI that may be due to other viral infections. These could be several viruses are known to cause respiratory infections in humans [39]. Major pathogens that induce URIs are human rhinoviruses (HRVs), belongs to *Picornaviridae* [40]. Other common causative agents are adenovirus, parainfluenza virus, enterovirus (EV) from the family Picornaviridae and genus Paraechovirus, coronavirus, and human Bocavirus [41, 42]. While the major bacterial agents responsible for RTIs have remained the same for the past 20–30 years, including: *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus*, beta-haemolytic Group A *Streptococcus* and *Pseudomonas* [43].

According to the age of infected children, the cases were categorized into three groups (Fig. 2). The mean age of hMPV and RSV was 33.28±5.3 and 19.93±4.8 month, respectively and more than 60% of infected cases were under 3 year old children. This represented the highest significant difference of hMPV infection in young children other age groups (Table 1). Although the RSV cases observed in children less than 2 years, there is no significant difference among infected children.

Table 1: Appearance of distribution viral infections according to age groups of children.

Genus of virus	Age/months	No. of case	Mean±SE	X^2-test	P-value
hMPV	1-24	38	12.84±1.056	27.97	0.000
	25-60	13	40.77±2.381		
	61-180	7	130.29±13.205		
RSV	1-24	9	8.33±2.466	1.923	0.166
	25-60	4	39±5.196		
	61-180	---	------		

Again results of our study showed that most patients with viral infection were younger than 5 years (91.2%), which is a similar result were reported in previous studies [44]. Epidemiology data showed that hMPV is responsible for approximately 5-8% of acute respiratory infection hospitalizations and 2-6% of community ARI cases in the under-5 years in industrialized countries [27].

Many researchers proved that almost all children become infected with RSV during their first or second year of life and that the incidence of RSV bronchiolitis reaches a maximum at the age of 2 months [45, 46].

The higher frequency of viral infection that cause URTI in young children could be due to one or multiple factors such as: immune system immaturity of young children; physiological population and cultural characteristics; rapid inflammatory responses; functional immaturity of the Eustachian tube and close contact with other children either directly, for example at school, or indirectly through relatives or common places [43].

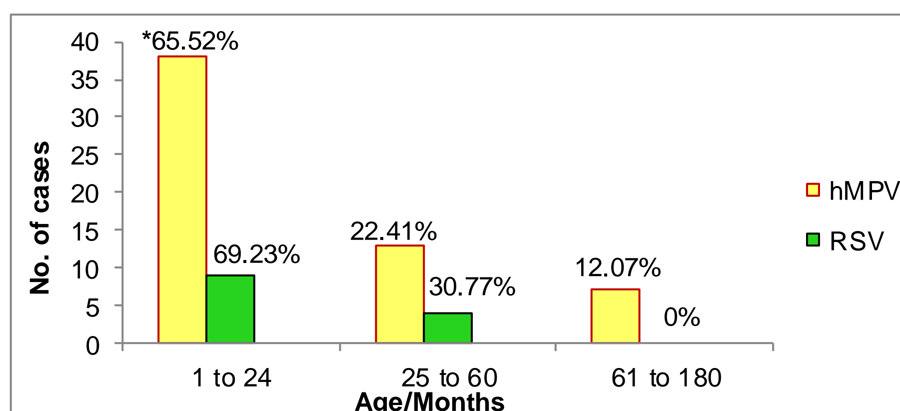

Figure 2: Relation of viral infection to age groups of infected children.

On the other hand, the frequency of hMPVs among the months showed high significant fluctuation (X^2=26.69, P<0.001) likely in Oct, Nov and Jan months (Fig. 3). But nearly spring and late winter months, especially Feb and Mar showed the highest percentage of the RSVs infection (seasonal infection) with slightly statistical significant differences between them (X^2=5.692, P=0.05). This result supported by other investigators [47, 48]. Furthermore, this finding showed that hMPV occurrence more 50% of total cases in those months, while the RSV present more than 60% during Feb month. The seasonal distributions of hMPV and RSV have been shown to overlap winter epidemics; therefore potential for dual infection exists. Several studies have found co-infection rates greater than 10% [49]. Meanwhile, other research observed the co-infections with these two pathogens are not unlikely to occur, but their clinical importance requires more research [50].

Infections with respiratory viruses exhibit distinct seasonal patterns in most temperate regions. Typically, RSV and influenza cause annual recurrent well-defined epidemics during the cold months [51]. The activity of hMPV has been shown to be greatest in winter and spring in the northern hemisphere [27] and autumn through spring in the southern hemisphere [52].

Different studies have shown that RSV infections peaked during the cold months in temperate regions in the southern hemisphere, seemingly independent of rainfall. In sub-tropical and tropical locations with seasonal rainfall, RSV tends to occur in relation to the rainy season, however, in locations closer to the equator with perennial rainfall, RSV activity is almost continuous and with varying peaks of infection [53]. In USA, most RSV infections occur during a period of about 22 weeks from November to May, with the peak being in January and February [54]. Other study showed that the disease was most prevalent and clearly active during autumn and early winter [55].

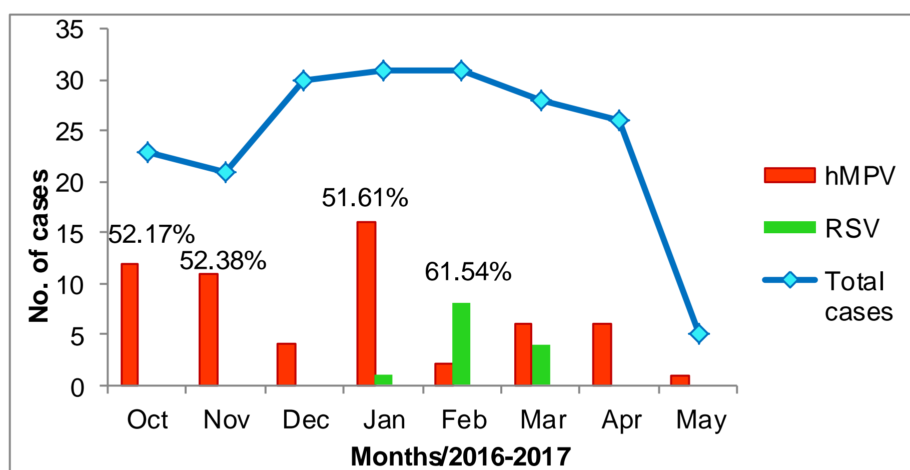

Figure 3: Distribution of pneumoviral infections along with total cases in months during 2016-2017

4. CONCLUSION

The present study suggested that performing the multiplex RT-PCR assay to report the molecular epidemiology of Paramyxoviridae in respiratory tract along with influenza virus during flu season.

5. REFERENCES

1. Madhi, S A., Levine, O.S., Hajjeh, R., Mansoor, O.D. and Cherian, T. (2008) Vaccines to prevent pneumonia and improve child survival. *Bull World H. Organ*. 86:365-372.
2. Bertino JS. (2002). Cost burden of viral respiratory infections: issues for formulary decision makers. Am J Med.; 112:42S–49S.
3. Barends, M., M. van Oosten, C. G. H. de Rond, J. A. M. A. Dormans, A. D. M. E. Osterhaus, H. J. Neijens, and T. G. Kimman. (2004). Timing of Infection and Prior Immunization with Respiratory Syncytial Virus (Rsv) in Rsv-Enhanced Allergic Inflammation. The J Infect Dise*.*, 189(10): 1866-72.
4. Cane, Patricia A. (2001). Molecular Epidemiology of Respiratory Syncytial Virus. John Wiley & Sons, Ltd. Pp. 103-16.
5. Zlateva, Kalina T., Philippe Lemey, Elien Moes, Anne-Mieke Vandamme, and Marc Van Ranst. (2005). Genetic Variability and Molecular Evolution of the Human Respiratory Syncytial Virus Subgroup B Attachment G Protein. J. Virol. 79(14): 9157-67.
6. Bossart, Katharine N, and Christopher C Broder. (2000). *Paramyxovirus Entry*: Madame Curie Bioscience Database [Internet]. Austin (TX): http://www.landesbioscience.com/curie/.
7. Barbosa Ramirez J, Pulido Dominguez P, Rey Benito G, Mendez Rico J, Castellanos J, Páez Martinez A. (2014). Human respiratory syncytial virus and metapneumovirus in patients with acute respiratory infection in Colombia, 2000-2011. Revista Panamericana de Salud Pública., 36(2):101–9.
8. Panda S, Mohakud NK, Pena L, Kumar S. (2014). Human metapneumovirus: review of an important respiratory pathogen. Int J Inf Dis*.*, 25: 45–52.
9. Falsey, AR., Erdman, D., Anderson, L.J. and Walsh, E.E. (2003). Human metapneumovirus infections in young and elderly adults. J. Infect. Dis. 187:785–790.
10. Principi, N., Bosis, S. and Esposito, S. (2006) Human metapneumovirus in paediatric patients. Clin. Microbiol. Infecti. 12:301-308.
11. Aberle, J. H., S. W. Aberle, M. Redlberger-Fritz, M. J. Sandhofer, and T. Popow-Kraupp. (2010). Human metapneumovirus subgroup changes and seasonality during epidemics. Pediatr. Infect. Dis. J. 29:1016–1018.
12. Aberle, S. W., J. H. Aberle, M. J. Sandhofer, E. Pracher, and T. Popow- Kraupp. (2008). Biennial spring activity of human metapneumovirus in Austria. Pediatr. Infect. Dis. J. 27:1065–1068.
13. Beneri, C., C. C. Ginocchio, R. Manji, and S. Sood. (2009). Comparison of clinical features of pediatric respiratory syncytial virus and human metapneumovirus infections. Infect. Control Hosp. Epidemiol. 30:1240–1241.
14. Williams, J. V., et al. (2004). Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N. Engl. J. Med. 350:443–450.
15. Williams, J. V., et al. (2006). The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. J. Infect. Dis. 193:387–395.
16. Fadhil, H.Y. (2015). Real-Time PCR detection of respiratory syncytial virus (RSV) among Adults with Pneumonia. Iraqi Journal of Science, 56(2C): 1617-1621.
17. Boyce, T.G., Mellen, B.G., Mitchel, E.F., Wright, P.F., Griffin M.R. (2000).Rates ofhospitalization for respiratory syncytial virus infection among children in Medicaid.J.Pediatr., 137:865–70.
18. Akhter, J. and Al Johani, S. (2011). Epidemiology and Diagnosis of Human Respiratory Syncytial Virus Infections, Human Respiratory Syncytial Virus Infection, Dr. Bernhard Resch (Ed.).
19. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA (2003). Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis, 9(3): 372-5.
20. Cote, S., Abed, Y. and Boivin, G. (2003). Comparative evaluation of real-time PCR assays for detection of the human metapneumovirus. J. Clin. Microbiol. 41:3631-3635.
21. Ebihara, T., Kikuta, H., Ishiguro, N., Ishiko, H. and Kobayashi, K. (2004). Comparison of the seroprevalence of human metapneumovirus and human respiratory syncytial virus. J. Med. Vir. 72:304–306.
22. Leung, J., Esper, F., Weibel, C. and Kahn, J.S. (2005). Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. J. Clin. Microbiol. 43:1213–9.
23. Kim C, Ahmed JA, Eidex RB, Nyoka R, Waiboci LW, Erdman D, et al. (2011). Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One., 6(6):e21610.
24. Mentel, R., Wegner, U.,Bruns, R., Gurtler, L. (2003). Real-time PCR to improve the diagnosis of respiratory syncytial virus infection.*J. Med.Microbiol*., 52:893–896.
25. IBM Corporation, USA. https://www.csun.edu/sites/default/files/statistics20-briefguide-64bit.pdf.
26. Saleh D.S., Fadhil H.Y. and Al-Hamdani F.G. (2012) Screening for High-Risk Human Papillomavirus by Real-Time PCR and Its As-sociation with Cytological Abnormalities of Pap Smears

in Baghdad. International Journal of Microbiology Research, 4(9): 332-335.

27. Van den Hoogen BG, Osterhaus DM, Fouchier RA.(2004). Clinical impact and diagnosis of human metapneumovirus infection. Pediatr Infect Dis J., 23:S25–S32.
28. Miller, S. A., Tollefson, S., Crowe, J. E. Jr., Williams, J. V., Wright, D. W. (2007). Examination of a fusogenic hexameric core from human metapneumovirus and identification of a potent synthetic peptide inhibitor from the heptad repeat 1 region. *Journal of Virology*, 81: 141-9.
29. De Graaf, M., Osterhaus, A. D., Fouchier, R. A., Holmes, E. C. (2008). Evolutionary dynamics of human and avian metapneumoviruses. *Journal of General Virology*, 89: 2933-42.
30. Fadhil, H.Y. (2014). Detection for respiratory syncytial virus RNA among children with Flu-like illness using molecular assay. International Journal of Current Research, 6(11): 9455-9459.
31. Al-Mossawi, M.M.K., Al-Hamadani, A.H. and Al-Hilali, H.A. (2017). Molecular Investigation of *Human metapneumovirus* and *Respiratory syncytial virus* in Children in Al-Amarah City. University of Al-Qadisiayah, College of Medicine, Department of Medical Microbiology, pp.163.
32. Al-Mola, G.A., Ragheb, A. and Abass, I.R. (2013). Human Metapneumovirus (hMPV) associated with respiratory infection in children hospitalized with acute lower respiratory tract infection in Hilla, Iraq. International Journal of Disease and Disorder, 1(2): 020-023.
33. Aziz, T.A.G. (2015). Detection of Human Metapneumovirus in Hospitalized Children with Acute Respiratory Tract Infections in Sulaimani Province, Iraq. Med Microb Diagn, 4(2):1-5.
34. Moattari, A., Aleyasin, S., Emami, A., Fyruzi, M. and Pirbonyeh, N. (2015). The Prevalence of Human Metapneumovirus and Respiratory Syncytial Virus and coinfection with both in hospitalized children with acute respiratory infection in South of Iran. Arch Pediatr Infect Dis., 3(3): e21581.
35. Paranhos-Baccala G, Komurian-Pradel F, Richard N, Vernet G, Lina B, Floret D. (2008). Mixed respiratory virus infections. *J Clin Virol.,* 43(4):407–10.
36. Al-Sonboli N, Hart CA, Al-Aeryani A, Banajeh SM, Al-Aghbari N, Dove W, et al. (2005). Respiratory syncytial virus and human metapneumovirus in children with acute respiratory infections in Yemen. Pediatr Infect Dis J., 24(8):734–6.
37. McCracken JP, Arvelo W, Ortiz J, Reyes L, Gray J, Estevez A, et al. (2014). Comparative epidemiology of human metapneumovirus- and respiratory syncytial virus-associated hospitalizations in Guatemala. Influenza Other Respir Viruses., 8(4):414–21.
38. Barbosa Ramirez J, Pulido Dominguez P, Rey Benito G, Mendez Rico J, Castellanos J, Páez Martinez A. (2014). Human respiratory syncytial virus and metapneumovirus in patients with acute respiratory infection in Colombia, 2000-2011. Revista Panamericana de Salud Pública*.,* 36(2):101–9.
39. Eccles, R. (2005). Understanding the symptoms of the common cold and infl uenza. Lancet Infect Dis., 5: 718-725.
40. Fendrick, A.M. (2003). Viral Respiratory Infections Due to Rhinoviruses: Current Knowledge, New Developments. A.M. J .Th er., 10: 193-202.
41. Passioti, M., Maggina, P., Megremis, S. and Papadopoulos, N.G. (2014). The common cold: potential for future prevention or cure. Curr Allergy Asthma Rep., 14: 413.
42. Khalaf, R.J., Fadhil, H.Y., Aufi, I.M., Salah Ali Namdar, S.A. (2017). Molecular diagnosis of Human Adenovirus in children with upper respiratory tract infections. J Pharm and Biolo Sciences,12(3): 09-13.
43. Mansilla, E., Ciceran, A., Pignatari, S., Koatz, A. M. and Marengo, R. L. (2016). The place of immunomodulation in recurrent respiratory tract infections, an overlooked pandemic. EMJ Respir., 4(1): 3-9.
44. Hata ,M., Ito, M. and Kiyosawa, S. (2007). A fatal case of encephalopathy possibly associated with human metapneumovirus infection. Japanese J. Infect. Dis., 60**:**328–329.
45. Reiche, Janine, and Brunhilde Schweiger. "Genetic Variability of Group a Human Respiratory Syncytial Virus Strains Circulating in Germany from 1998 to 2007." *J. Clin. Microbiol.* 47, no. 6 (2009): 1800-10.
46. Dapat, Isolde C., Yugo Shobugawa, Yasuko Sano, Reiko Saito, Asami Sasaki, Yasushi Suzuki, Akihiko Kumaki, Hassan Zaraket, Clyde Dapat, Taeko Oguma, Masahiro Yamaguchi, and Hiroshi Suzuki. "New Genotypes within Respiratory Syncytial Virus Group B Genotype Ba in Niigata, Japan." *J. Clin. Microbiol.* 48, no. 9 (2010): 3423-27
47. Weigl, J.A.I., Puppe, W. and Meyer, C.U. (2007) Ten years' experience with yearround active surveillance of up to 19 respiratory pathogens in children. *European J. Pediat.***166**:957-966.
48. Frogel, Michael P., Dan L. Stewart, Michael Hoopes, Ancilla W. Fernandes, and Parthiv J. Mahadevia. "A Systematic Review of Compliance with Palivizumab Administration for Rsv Immunoprophylaxis." *Journal of Managed Care Pharmacy* 16, no. 1 (2010).
49. Viazov, S., Ratjen, F., Scheidhauer, R., Fiedler, M. and Roggendorf, M.(2003). High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J. Clin. Microbiol.41:3043-3045.
50. Rawlinson WD, Waliuzzaman Z, Carter IW, et al. (2003). Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. J Infect Dis., 187:1314–1318.
51. Stensballe, L.G., Devasundaram, J.K. and Simoes, E.A. (2003) Respiratory syncytial virus epidemics: the ups and downs of a seasonal virus. Pediatr. Infect. Dis. J., 22:S21- 32.
52. Lambert, S.B., Allen, K.M. and Druce, J.D. (2007). Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy pre-school aged children using parent-collected specimens. Pediatrics. 120:929-937.
53. Weber, M.W., Mulholland, E.K. and Greenwood, B.M. (1998). Respiratory syncytial virus infection in tropical and developing countries. Tropical Med. Internat. Health 3:268–280.
54. Hall, Caroline B. (2001). Respiratory Syncytial Virus and Parainfluenza Virus. The New England Journal of Medicine*,* 344(25): 1917-28.
55. Shobugawa, Yugo, Reiko Saito, Yasuko Sano, Hassan Zaraket, Yasushi Suzuki, Akihiko Kumaki, Isolde Dapat, Taeko Oguma, Masahiro Yamaguchi, and Hiroshi Suzuki. (2009).Emerging Genotypes of Human Respiratory Syncytial Virus Subgroup among Patients in Japan. J. Clin. Microbiol., 47(8): 2475-82.

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