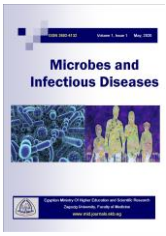
Microbes and Infectious Diseases 2022; 3(2): 279-285



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Sequencing of rhinoviruses in Egyptian children with respiratory tract infections

Mona A. Khattab [*1], Shereen El Sayed Taha [1] , Fatma M Abd El Aziz [2] , Nancy M Abu Shady [3], Rachel Scheuer [4], Ron AM Fouchier [4], Ali M Zaki [1]

1- Department of Medical Microbiology and Immunology Faculty of Medicine, Ain Shams University, Cairo, Egypt.
2- Department of Medical Microbiology and Immunology Faculty of Medicine, Misr University for Science and Technology, Giza, Egypt.
3- Department of Pediatrics Faculty of Medicine, Ain Shams University, Cairo, Egypt.
4- Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands.

ARTICLE INFO

Article history:
Received 9 December 2021
Received in revised form 16 January 2022
Accepted 18 January 2022

Keywords:
Human rhinoviruses (HRV)
PCR
Viral proteins (VP)

ABSTRACT

Background: Human rhinoviruses (HRV) are one of the most common causes of upper respiratory tract infections among young children. Human rhinoviruses have a wide genetic diversity. They include three different species A, B and-C. Acute Respiratory Infection (ARI) is considered to be an important cause of morbidity and mortality in neonates and children. **Aim of the study:** To detect the common subtypes of circulating HRV among Egyptian children with respiratory infections for further epidemiological characterization. **Methods:** We enrolled 161 children admitted to Ain Shams Pediatric University Hospital complaining of respiratory tract infections. Human rhinoviruses were detected by RT-PCR. Sequencing of HRV was done based on viral proteins (VP4-VP2) genomic region analyses by RT-PCR. **Results:** HRV were detected in 54 cases (33.5%) with respiratory tract infections. Sixty-five **(**65) % of detected HRV was in children aged 5-10 years. Molecular sequencing showed high prevalence of HRV-C (67%) followed by HRV-A (33%). **Conclusion:** This study is from the first few studies that revealed diversity of HRV in Egypt. Different phylogenetic studies are needed to evaluate their diversity and to trace their spread and epidemiological origin.

Introduction

Human rhinoviruses (HRV) are known as small non-enveloped RNA viruses. They belong to picornaviruses [1]. They are one of the most common causes of upper respiratory tract infections among young children aged less than 5 years [2]. Acute Respiratory Infection (ARI) is considered to be one of the most important causes of morbidity and mortality in neonates and children below 5 years where the estimated deaths annually is 4.2 millions [3]. Furthermore, HRV can cause infections ranging from bronchiolitis to pneumonia and exacerbation of asthma [4]. Moreover, early infection with HRV can cause damage to airways and exacerbation of asthma [5]. HRV have a wide genetic diversity. They include three different species A, B and-C, which were subdivided into 169 types [1]. Human rhinoviruses -A was the most diverse, it includes 80 types, however, HRV-B includes 32 types. Moreover with the advance of molecular technology, HRV-C was discovered in 2006 and divided into 57 types [6]. The viral protein (VP4)/VP2 coding region plays an important role in type identification based on its discriminatory potential [7]. Furthermore, HRV species are

DOI: 10.21608/MID.2022.113594.1222
* *Corresponding author:* Mona A. Khattab
E-mail address: monaadelhkattab@med.asu.edu.eg

distributed globally and also found in all age groups. However, there is a little known in African and Middle Eastern regions about circulating types [8].

Aim of the study

To detect the common subtypes of circulating HRV among Egyptian children with respiratory infections for further epidemiological characterization.

Patients and Methods

This study was conducted on 161 children admitted to Ain Shams Pediatric University Hospital complaining of respiratory tract infections during the period of December 2017 to January 2019. Their age ranges from 1-10 years with mean SD 5.48 ± 2.09. They were 73 (45.3 %) males and 88 (54.7%) females. 37 children (23 %) enrolled in this study were diagnosed to have upper respiratory tract infections (URT) while 124 children (77%) were diagnosed to have lower respiratory tract infections (LRT) . Informed consent was taken from their parents. Patients with chronic diseases as cystic fibrosis, congenital heart diseases and bronchopulmonary dysplasia were excluded from the study. Nasopharyngeal specimens were collected from each patient. Specimens were transported using transport medium and aliquots of samples were also stored at −80˚C for additional molecular investigation.

Detection of HRV by real time RT- PCR

Viral RNA was extracted using commercial RNA isolation procedure (Qiagen viral RNA extraction kit, Germany)

Real time RT-PCR

For detection of HRVs, One step real time RT-PCR was performed using the quantitect probe RT-PCR master mix (Qiagen Germany) in a total volume of 25 µl per sample. Each tube contained 12.5 µl of the ready master mix plus 1 µl of each primer and probe completed to 20 µl with PCR grade water and 5 µl of each sample. The following HRV specific primers and probe were used: RHINO F (5'AGC CTG CGT GGC KGC C 3'), RHINO R (5'GAA ACA CGG ACA CCC AAA GTA GT 3') and probe (Fam 5'CTC CGG CCC CTG AAT GYG GCT AA BHQ).

Real time RT-PCR was done using a One Step plus real time machine (Applied Biosystem,USA), using the subsequent reaction conditions: initial reverse transcriptase step at 50ºC for 30 minutes, then hot start enzyme activation at 95ºC for 15 minutes, followed by 40 cycles of 95ºC for 30 seconds, 60ºC for 1 minute. All runs include positive, negative, and no template controls. RT-PCR positive samples were identified automatically when fluorescence signal exceeds the threshold level determined by the machine. (Applied Biosystem real time machine USA. Soft ware applied biosystem sotware, USA).

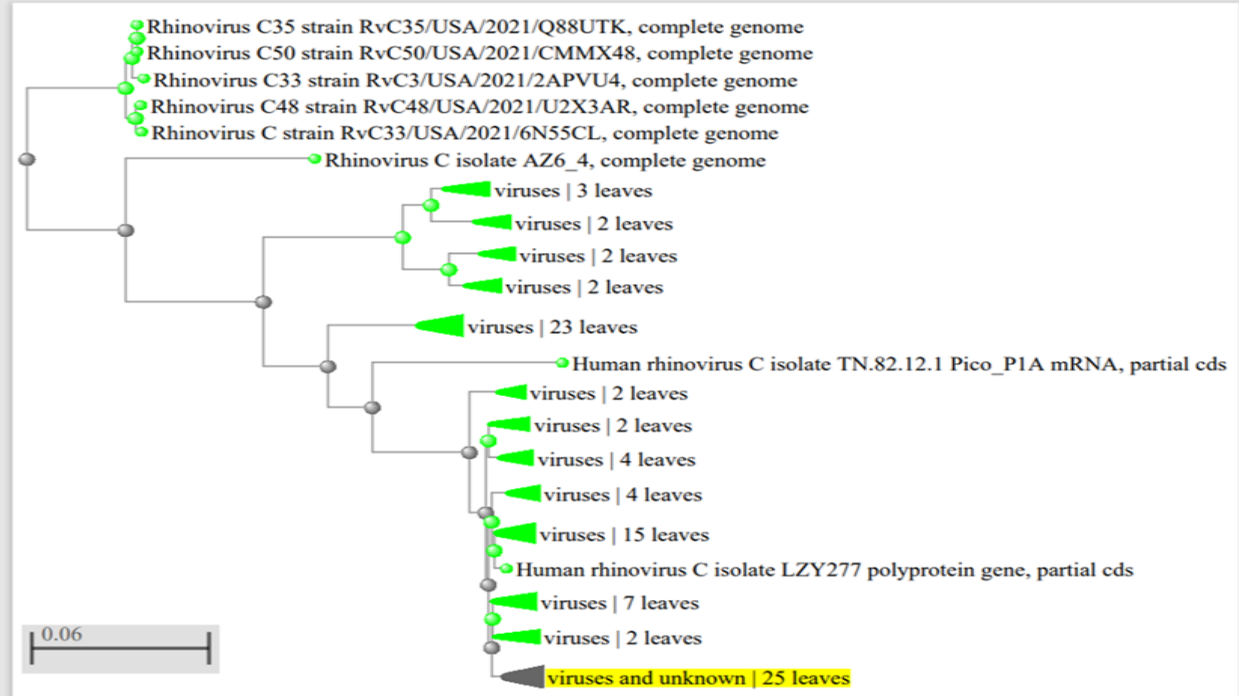
Sequencing of HRV

Twelve selected positive HRV samples were subjected to sequencing for detecting the common prevalent subtypes of HRV in Egypt. Identification of type was based on VP4/VP2 junction sequence analysis. The 5′UTR and VP4/VP2 regions (approximately 290 and 542 nucleotides, respectively) of selected HRV positive samples were amplified by RT-PCR. PCR for VP4/VP2 was done using a forward primer (5'-CGG CCC CTG AAT GYG GCT AA-3') and reverse primer (5'- TCN GGN ARY TTC CAV CAC CAN CC -3') and semi-nested PCR was done with a second forward primer (5'- CTA CTT TGG GTG TCC GTG TTT C-3') and the same reverse primer as in the first reaction. Initial PCR for the 5'UTR was performed using a primer (5'- CAA GCA CTT CTG TTT CCC CGG-3') and reverse primer (5'-GAA ACA CGG ACA CCC AAA GTA GT-3') and semi-nested PCR was done with the same forward primer as in the first reaction and a second reverse primer (5'- CAT TCA GGG GCC GGA GGA-3') according to **Linsuwanon et al.** [9].

The PCR products were purified from agarose gel and sequenced using Big Dye Terminator v3.1 (Applied biotechnology, Bleiswijk, Netherlands).

Sequencing was done done at Lab. At EMC Erasmus Medical Center , **Netherlands.** Genetic analyzer 3130XL (Applied Biosystem, Bleiswijk, Netherlands).

Figure 1. Phylogenic tree of HRVC.



Statistical analysis

All statistical analyses were performed using IBM SPSS. Quantitative variables were used as mean ± standard deviation (SD), numbers and percentages, the qualitative variables were used as Chi square test and t-test. Probability (*p*) value of less than 0.05 was considered significant.

Results

Demographic and clinical data

This study was conducted on 161 children admitted to Ain Shams Pediatric University Hospital complaining of respiratory tract infections during the period of 2017-2018. Their age ranges from 1-10 years with mean SD 5.48 ± 2.09. They were 73 (45.3 %) males and 88 (54.7%) females.

Human rhinoviruses was detected in 54 nasopharyngeal specimens (33.5%). Moreover, HRV was more detected in 72 % of acute bronchitis followed by 24% in other upper respiratory tract infections **(Table 2, Figure 2)**.

Furthermore, our study showed no statistically significant association of HRV detection with age or sex. However, the majority of HRV in children aged 5-10 years was 65% while in age group 2-4 HRV was found to be 28% and in infants aged 1 year HRV was 7**% (Table 3 &4. Figure 3)**.

Regarding genotypic distribution of the 12 selected HRV, we found 8 characterized as HRV-C (67 %) and 4 characterized as HRV-A (33%) **(Figure 4)**.

Table 1. Demographic and clinical data of children with respiratory tract infections.

Demographic and clinical data	
Sex Males, n (%); Females, n (%)	 73 (45.3 %); 88 (54.7 %)
Age Mean ± SD	 5.48 ± 2.09
Clinical presentation **Acute bronchitis n (%)** Other upper respiratory tract infections n (%) Typical pneumonia n(%) Atypical pneumonia n (%)	 103 (64 %) 37 (23 %) 6 (3.7 %) 15 (9.3%)

Table 2. Detection of HRV by RT-PCR in nasopharyngeal specimens.

Detection method		N	%
PCR	Negative	107	66.5
	Rhino virus	54	33.5
	Total	161	100.0

Table 3. Relation between gender with clinical diagnosis and HRV detected.

		Sex						Chi square test	*p* value
		Male		Female		Total			
		N	%	N	%	N	%		
Diagnosis	Acute bronchitis	45	43.7	58	56.3	103	100	1.23	0.75 (NS)
	Other upper respiratory tract infection	17	45.9	20	54.1	37	100		
	Pneumonia	4	66.7	2	33.3	6	100		
	Atypical pneumonia	7	46.7	8	53.3	15	100		
Infection with Rhinovirus	Negative	53	49.5	54	50.5	107	100	2.26	0.13 (NS)
	Positive	20	37.0	34	63.0	54	100		

Table 4. Association of HRV detection with age.

		Age			
		Minimum	Maximum	Mean	SD
Infection with Rhino virus	Negative	1.00	10.00	5.62	2.09
	Positive	1.00	9.00	5.20	2.10
	t* = 1.18 P value = 0.24 (NS)				

Figure 2. Distribution of HRV among clinical samples.

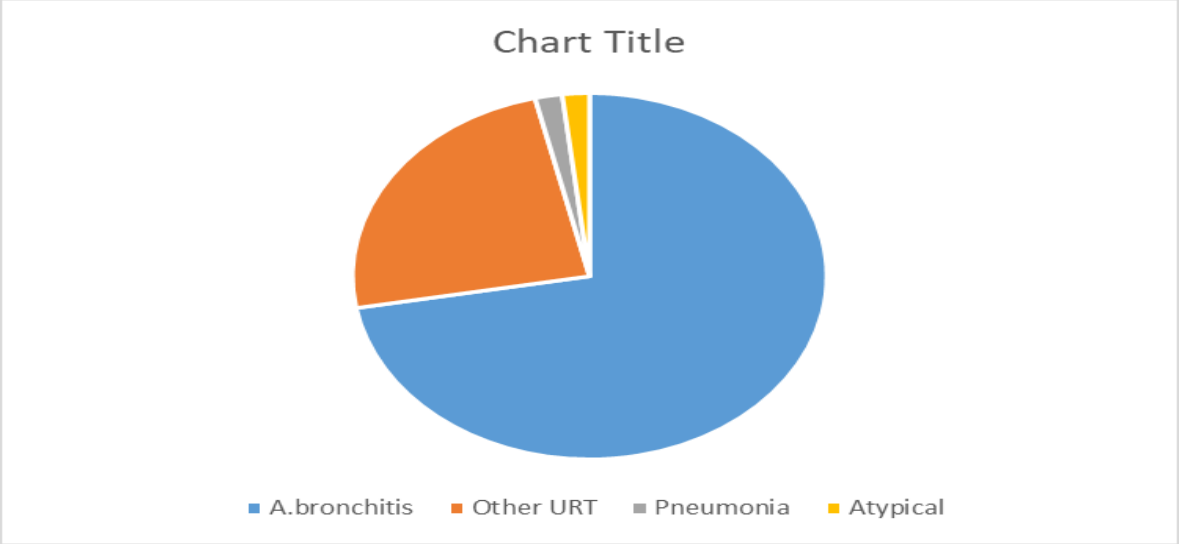


Figure 3. Distribution of HRV among different age groups **.**

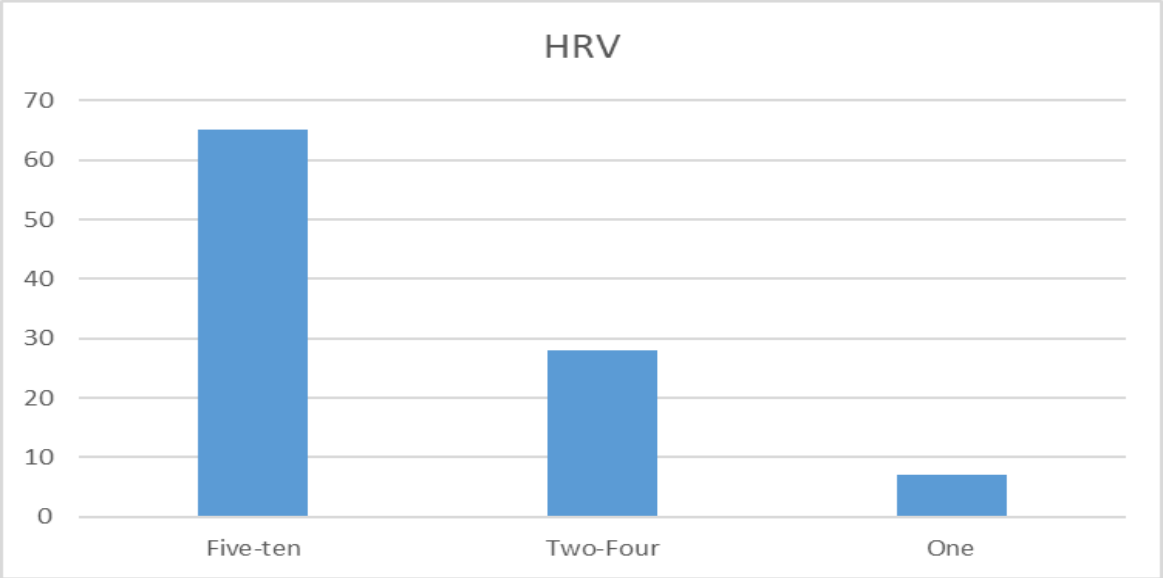
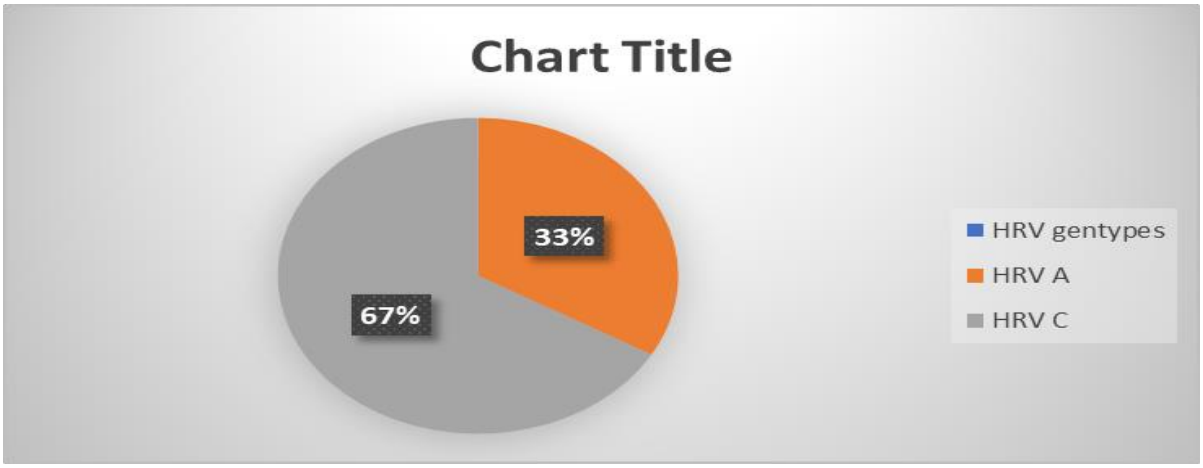


Figure 4. Distribution of HRV genotypes**.**



Discussion

Human rhinovirus is an important leading cause of ARI in infants and young children. However, it has diverse genotypic distribution world wide and a little is known in African regions. Thus, this study aims to detect the common sequence of circulating HRV among Egyptian children with respiratory infections for further epidemiological characterization.

In this study , HRV was detected in 33.5 % of nasopharyngeal specimens. This come in accordance with other studies that demonstrated a HRV prevalence of about 12-30% [10-12]. On the other hand, a study done by **Haddad-Boubaker et al.** [13] detected HRV in (61%) of cases with acute respiratory tract infections (ARTI) . However, in another study HRV was detected in 17.8% of children presenting to hospital with ARI [14]. These differences in detection rates may be explained by the specificity and sensitivity of the assay used and HRV epidemiological and demographic variabilities world wide.

In the current study, HRV was detected in 72 % of acute bronchitis followed by 24% in other upper respiratory tract infections and 4% in other lower respiratory tract infections. Furthermore, **Fawkner-Corbett et al.** [14] stated that most of children infected with HRV were presented with lower respiratory tract symptoms as bronchiolitis, pneumonia, and asthma.

In this study, there is insignificant statistical association of HRV detection with patient age or gender.

This variations in the distribution of HRV among different age group may be explained by cumulative acquired immunity to different serotypes

that cause reduction of infection rates in older children. Furthermore, younger children are more vulnerable to infection more than older children as their immune system immature yet. However, older children can also children experience an average range of two to three times RV infection per year [15].

Regarding the distribution of HRV genotypes, we found that 8 characterized as HRV-C (67 %) and 4 characterized as HRV-A (33%). We did not find HRV-B as it is often found at very low levels (0–3%) or in relatively asymptomatic patients. This was in accordance to other studies that did not find HRV-B [16,17]

However, a recent study done by **Haddad-Boubaker et al. [13]** showed that the HRV-A species was predominant (63.3%) followed by HRV-C (30.6%). Similar results were obtained among hospitalized children in different regions [18,19]

Moreover, in a study done by **Fawkner-Corbett et al. [14]** reported an association between HRV-C and severe ARI, particularly in young children as they presented with wheezing ARI. They concluded that in contrast to other HRV species, HRV-C grows optimally at both 34˚C or 37˚C [14].

Conclusions

Picornaviruses (HRV) considered to be a major cause of ARI in Egyptian children from five to ten years. To the best of our knowledge, this is one of the first studies describing the diversity of HRV types in Egypt providing more knowledge on HRV infections in those regions. However, deeply phylogenetic studies of different detected types may be helpful to identify precisely the diversity of HRV different types and try to trace their epidemiological origin for further epidemiological characterization.

Conflicts of interests: none.

Financial disclosure: none.

References

1- **International Committee on Taxonomy of Viruses:** The online 10th report of the International Committee on Taxonomy of Viruses. Available at: https://ictv.global/taxonomy. 2019. Accessed: 03 February 2021 .

2- **Jacques J, Bouscambert-Duchamp M, Moret H, Carquin J, Brodard V, Lina B, et al.** Association of respiratory picornaviruses with acute bronchiolitis in French infants. J Clin Virol 2006;35: 463-466.

3- **World Health Organization (WHO)**.The global burden of disease: 2004 update. Geneva WHO 2008. Available at: http:// www.who.int/healthinfo/global_burden_disease/GBD_report _2004update_full.pdf. Accessed: 03 February 2021.

4- **Cheuk DK, Tang IW, Chan KH, Woo PC, Peiris MJ, Chiu SS.** Rhinovirus infection in hospitalized children in Hong Kong: a prospective study. Pediatr Infect Dis J 2007;26: 995-1000.

5- **Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al.**Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Amm J Respir Crit Care Med 2008; 178(7):667-672

6- **Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, et al.**MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenzalike illness in New York State during 2004–2005. J Infect Dis 2006; 194: 1398–1402

7- **McIntyre CL, Knowles NJ, Simmonds P.** Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. J Gen Virol 2013; 94: 1791-1806.

8- **Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T.** Proposals for the classification of human rhinovirus species C into genotypically assigned types. J Gen Virol 2010; 91: 2409-2419

9- **Linsuwanon R, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch**

J, Theanboonlers A , et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. Journal of Infection 2009;59: 115-12.

10-**Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, et al.** Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004; 10:1095– 1101.

11-**Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, et al.** New Vaccine Surveillance Network.Rhinovirus associated hospitalizations in young children. J Infect Dis 2007;195:773–781.

12-**Wang W, Cavailler P, Ren P, Zhang J, Dong W, Yan H,et al.** Molecular monitoring of causative viruses in child acute respiratory infection in endemo-epidemic situations in Shanghai. J Clin Virol 2010; 49:211–218.

13-**Haddad-Boubaker S, Mefteh K, Mejri C, Bouaffsoun A , El Moussi A, Ilhem Boutiba I,et al.** High genotypic diversity of Rhinoviruses obtained from Tunisian children with severe acute respiratory infection. JIDC 2021;15 (5):726-735.

14-**Fawkner-Corbett DW, Khoo SK, Duarte CM, Bezerra PGM, Bochkov YA, Gern J E, et al.** Rhinovirus-C Detection in Children Presenting With Acute Respiratory Infection to Hospital in Brazil. J. Med. Virol 2016; 88:58–63.

15-**Chen J, Hu P, Zhou T, Zheng T, Zhou L, Jiang C, Pei X.** Epidemiology and clinical characteristics of acute respiratory tract infections among hospitalized infants and young children in Chengdu, West China, 2009-2014. BMC Pediatr 2018; 18: 216.1-8.

16-**Lau SKP, Yip CCY, Tsoi HW, Lee RA, So LY, Lau YL, et al.**Clinical features and complete genome chara cterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 2007; 45:3655–3664.

17-**Dominguez SR, Briese T, Palacios G, Hui J, Villari J, Kapoor V, et al.** Multiplex MassTag-PCR for respiratory pathogens in pediatric nasopharyngeal washes negative by conventional diagnostic testing shows a high prevalence of viruses belonging to a newly recognized rhinovirus clade. J Clin Virol 2008; 43:219–222.

18-**Ratnamohan VM, Zeng F, Donovan L, MacIntyre CR, Kok J, Dwyer DE.** Phylogenetic analysis of human rhinoviruses collected over four successive years in Sydney, Australia. Influenza Other Respir Viruses 2016; 10: 493-503.

19-**Smuts HE, Workman LJ, Zar HJ.** Human rhinovirus infection in young African children with acute wheezing. BMC Infect Dis 2011; 11: 65.1-8.