Journal of Clinical & Cellular Immunology

Gad et al., J Clin Cell Immunol 2017, 8:5
DOI: 10.4172/2155-9899.1000526

Research Article Open Access

Viral Infections in Egyptian Hospitalized Children With Acute Respiratory Tract Infections

Niveen M Gad[1*], Doaa Refaay[2], Naglaa M Gad[3] and Zakia A Z Mohamed[4]

[1]*Microbiology and Immunology Department, Faculty of Medicine, Banha University, Egypt*

[2]*Pediatric Department, Faculty of Medicine, Banha University, Egypt*

[3]*Clinical Pathology Department, Faculty of Medicine, Banha University, Egypt*

[4]*Clinical Pathology Department, Faculty of Medicine (for girls), Al Azhar University, Cairo, Egypt*

[*]**Corresponding author:** Niveen M Gad, Microbiology and Immunology Department, Faculty of Medicine, Banha University, Egypt, Tel: +201001383106; E-mail: niveengad@yahoo.com

Received date: October 10, 2017; **Accepted date:** October 30, 2017; **Published date:** November 07, 2017

Abstract

Acute respiratory tract infections (ARTIs) are associated with significant morbidity and mortality worldwide, especially in developing countries. Children under the age of 5 years old are mostly affected. Both viral and bacterial causes are implicated, but viral etiologies are more difficult to diagnose. Viral infections can occur either single or mixed, but the significance and association of polyviral agents with the severity of cases are poorly understood. More studies are critical for understanding this role, improving diagnosis and treatment.

Objective: To identify the rule of five chosen viral pathogens in ARTIs in children below 5 years old and the association of polyviral etiology with the severity of the disease.

Methods: Nasopharyngeal swabs were taken from 120 children who had symptoms and signs of ARTIs, attending the outpatient clinic and admitted in pediatric department or pediatric intensive care unit, Banha university hospital. All children were subjected to full history taking, analysis by multiplex PCR for five viruses (Rhinovirus, Respiratory syncytial virus, Human metapneumovirus, Adenovirus, and Human boca virus).

Results: 54 viruses were identified by multiplex PCR from 41 children (34.2%), 75.6% of them had a single viral infection, (17% and 7.4%) were co-infected with two and three viruses respectively. Positive cases were mostly of children admitted in pediatric ward and the majority were infants. URTIs was the most common presentation followed by bronchiolitis then bronchopneumonia. HRV was the most frequently detected as a single and mixed infection (35.2%) followed by RSV (22.2%). Non-significant correlation was found between mixed infection and the severity of infection.

Conclusion: HRV is the most frequently recognized viral pathogen either as single or mixed in children below 5 years old in our patient group. Mixed infection has no correlation with disease severity. And using Multiplex PCR is an ideal tool for investigating mixed infection.

Keywords: ARIs; RSV; hRV; hMPV; ADV; hBoV; Multiplex PCR; Mixed infection

Introduction

Acute respiratory infections (ARIs) are the principle cause of hospitalization and death of infants and young children in developing countries [1]. Viral pathogens are mostly claimed in ARIs, including Influenza virus (Flu), Human parainfluenza virus (HPIV), Respiratory syncytial virus (RSV), Adenovirus (ADV), and Rhinovirus (RV). Moreover, the significance of newly recognized viruses such as Human metapneumovirus (HMPV), Human bocavirus (HBoV), and another two novel closely related Human polyomaviruses; KIV [2] and WUV [3,4], have been more evident.

Viral ARIs can occur as single or mixed microbial infections, but the significance of these polymicrobial infections has to be investigated. Therefore, accurate diagnosis of viral ARTI's has been shown to reduce the misuse of antibiotics and shorten the duration of hospital stay. Multiplex PCR has readily allowed for the simultaneous detection of pathogens in respiratory specimens. However the interpretation of these studies is confused by the differences of the respiratory pathogen included in the panel of analysis, and the patient population being studied [5]. In this study we chose five viruses to be analyzed including RSV, RV, HMPV and HBoV and AdV. Respiratory syncytial virus is RNA virus of the family *paramyxoviridae* and the most common cause of bronchiolitis and pneumonia in young children [6]. Human metapneumovirus, is another RNA virus of the same family, subfamily *Pneumoviridae* and the second most common cause of lower respiratory tract infections (LRTIs) of young children. It has been identified also as a significant cause of upper respiratory tract infections (URTI) in young children [7]. Rhinovirus, is RNA virus of the family *picornaviridae* and it's the most common cause of common cold, but it also can cause LRTIs [8].

Human bocavirus is a recently identified DNA viral agent that belongs to the family *Parvoviridae*. First isolated in 2005 and may persist and be associated with long-term diseases like lung fibrosis and cancer. Several studies have reported the prevalence of Boca infection worldwide as ranging from 2 to 21.5%, mainly in children younger than 3 years of age in whom the virus has been associated with URTI and LRTI. Currently, hBoVs are classified into species 1 through 4; HBoV1 is predominantly found in the respiratory tract [9,10].

Adenovirus, is DNA virus of the family *Adenoviridae* causing acute respiratory infection predominantly of types 1, 2, 5, 6, occasionally 3 and 7 and account for about 10% of viral respiratory infections. The contagiousness of the virus is related to the virus load [11].

Our objectives were to study the etiology and clinical associations of respiratory viruses in children with ARTIs under 5 years old and to investigate the association of polymicrobial infection with clinical severity.

Methodology

This study was a cross-sectional study carried in Pediatric and Microbiology and Immunology Departments, Faculty of Medicine, Banha University, in the period from November 2015 to March 2016. The study was conducted on 120 children with acute respiratory infection visiting pediatric clinic or admitted to Pediatric Department, Banha university Hospital. Their age ranged from 2 month to 5 years. All children were subjected to: full history taking from their parents, complete clinical examination, routine complete blood picture, C-Reactive Protein (CRP) analysis and chest X-ray examination. Cases with positive bacterial cultures were excluded.

Sample Collection

Nasopharyngeal swabs were collected from the children, immediately preserved in virus transport medium (VTM) [12] and stored at −80°C prior to testing. The study was approved by the local ethics committee of Banha University Hospitals and written consent was taken from each parent participant [13-16].

Sample Processing

(1)Nucleic acids extraction and purification

Genomic nucleic acid extraction was performed using The Thermo Scientific Gene JET Viral DNA and RNA Purification Kit (Thermo Fisher scientific, Australia). The kit utilizes silica based membrane technology in the form of a convenient spin column.

The extracted DNA concentration was detected through measurement by UV spectrophotometer. Readings were taken at wave lengths of 260 and 280 nm. Purified viral nucleic acid samples were immediately divided into 2 tubes one of them was stored at −20°C and the other was immediately used for reverse transcription.

(2) Reverse transcription

For RNA viruses, cDNA was obtained by using an efficient, rapid and DNA cleaning up reverse transcription system (FastQuant RT kit with gDNAse (TiangenBiotech,China).

Ten microliters of a mixture for cleaning up genomic DNA containing (2 µl of 5x g DNA Buffer, 2 µl of the sample and 6 µl RNase free H_2O) was added to 10 µl of master mix for reverse transcription containing (2 µl of 10x Fast RT Mix, 1 µl of RT Enzyme Mix, 2 µl of FQ-RT Primer Mix and 5µl of RNase free H_2O) and they were mixed by vortex.

The mixture was incubated for 15 min at 42°C followed by incubation for 3 min at 95°C. The obtained cDNA was stored at -20°C.

(3)Nucleic acid amplification:

Two PCR sets were performed one is a multiplex PCR for amplification of the purified genomic DNA of DNA viruses (ADV and HBoV), the other is a multiplex RT-PCR for amplification of cDNA of RNA viruses (HMNV, HRSV and Rhino virus).

Virus	Targetet	Primer	Sequence (5'–3')	PCR product size(bp)
1.Adenovius	VP6	Hex1deg-(F)	GCCSCARTGGKCWTACATGCACATC	300
	VP6	Hex2deg-(R)	CAGCACSCCICGRATGTCAAA	
2. HBoV	VP1/2	Boca-AK-VP-(F)	GGCTCCTGCTCTAGGAAATAAAGAG	500
	VP1/2	Boca-AK-VP-(R)	CCTGCTGTTAGGTCGTTGTTGTATGT	
3. HRSV		FI subunit of fusion (F) protein-(F)	TTAACCAGCAAAGTGTITAGA	243
		FI subunit of fusion (F) protein-(R)	TITTGTTATAGGCATATCATTG	
4. HMPV		L gene-(F)	CACCCCAGTCTTTCTTGAAA-	171
		L gene-(R)	CATGCCCACTATAAAAGGTCAG	
5. HRV		5NCR-VP4/VP2-(F)	CTCCGGCCCCTGAATRYGGCTAA	110
		5NCR-VP4/VP2-(R)	TCIGGIARYTTCCASYACCAICC	

Table 1: Primers and amplified product length for PCR and RT-PCR used for detection of the targeted respiratory viruses [13-16].

The reaction was performed in a final volume of 50 µL consisting of 25 µL of Maximum Hot Start Green PCR Master Mix (2x) (Thermo Scientific, Australia), 5 µL of template DNA or the cDNA was added to its corresponding PCR tube, 2.5 µL (1.0 µM) of each primer (forward and reverse) specific for each viral genome in each PCR set (Fermentas, Germany), water (nuclease free) to a final volume of 50 µL. All reagents were prior vortexed, and finally 25 µL of mineral oil were added to the reaction mixture. The primers used in amplifications for PCR or reverse transcription (RT)-PCR for each of the targeted viral agents are described in table 1. The selected primers were targeted to conserved regions of viral genomes.

Reactions were carried out in Thermal Cycler (Biometra, Germany). Amplification cycles of multiplex PCR were: Initial denaturation step at 95°C for 4 min, forty repeated cycles of: denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 30 s followed by final extension step at 72°C for 15 min then hold at 4°C. Amplification cycles of multiplex RT-PCR were: Initial denaturation step at 95°C for 4 min, forty repeated cycles of: denaturation at 95°C for 30 s, annealing at 40°C for 30 s and extension at 72°C for 60 s followed by final extension step at 72°C for 15 min then hold at 4°C.

10 µL of each amplified DNA and 100 bp ladder (molecular weight marker) (Fermentans, Germany) were separated on 1.5% agarose gel containing 0.3 µg/ml of ethidium bromide. The bands were visualized using UV transilluminator (Biometra, Germany) (254 nm).

Result

They were 66 males (55%) and 54 females (45%) and their age ranged between (2 months-5 years) old with mean of 1.45. Most of them were under empirical antibiotic treatment. Nasopharyngeal swabs were obtained from all cases and subjected to multiplex PCR for five viruses (RV, RSV, HMPV, ADV and HBoV).

	No. (%)
Sex: Male	25 (61)
Female	16 (39)
Source : Outpatient clinic	12 (29.2)
Ward	19 (46.3)
PICU	10 (24.4)
Age group: 0-12 m	10 (24.4)
13-24 m	16 (39)
25-36 m	7 (17)
37-48 m	5 (12)
49-60 m	3 (7.3)
Presentation: URTIs	14 (34.1)
Bronchitis	4 (9.7)
Bronchiolitis	12 (29.2)
Bronchopneumonia	8 (19.5)
Pneumonia	3 (7.3)

Table 2: Demographic data and presentation of 41 cases with positive PCR result

Out of 120 cases included, 41 (34.2%) were positive by multiplex PCR for different viral infections. 25 (61%) were males and 16 (39%) were females. Infants (≤ 2 yrs) were the most commonly affected age group (26 cases). URTIs was the most common presentation followed by bronchiolitis then bronchopneumonia. 29 children were admitted to the hospital (19 in pediatric ward and 10 in pediatric intensive care unit (PICU)). 12 children were from Pediatric Out patients Clinic (Table 2). 54 viruses were revealed from 41 children. 31 children (75.6%) had single viral infection, 7 (17%) were co-infected with two respiratory viruses, and 3 (7.4%) were co-infected with three respiratory viruses (Table 3). Positive cases were mostly from infants (33 viruses).

Respiratory virus	Positive Cases	No. of isolated viruses
Single viral isolation:		
RV	11	11
RSV	6	6
HMPV	7	7
ADV	5	5
HBoV	2	2
Double viral isolation:		
RV&RSV	2	4
RV&ADV	1	2
RSV&HBoV	1	2
RV&HMPV	2	4
ADV&RSV	1	2
Triple viral isolation		
RV&ADV&RSV	2	6
RV&HBoV&HMPV	1	2
Total	41	54

Table 3: Distribution of respiratory virus infections among 41 positive cases.

Discussion

This study was conducted to identify the viral etiologies of ARIs in children younger than five years old in relation to five viruses (RV, RSV, hMPV, ADV and HBoV), and the correlation of polymicrobial infection to severity of clinical outcome. Viral etiology was confirmed by PCR in 41 cases (34.2%) of 120 children; single virus was retrieved from 25.8% of them and 8.3% were coinfected with 2 or 3 viruses. This is less than other studies in which single viral etiology was isolated from 36-80% of studied groups, but It's near or less than other studies in which mixed viral etiologies were isolated from 6-40% of patients

[1,5,17,18]. A study of Cuti and his team mentioned that coinfection is reportedly related to the time of year when circulations of multiple viruses occur, and that there could be likely interplay between climatic, environmental and immunity level that contribute to viral coinfection [5]. The most common age affected by infection in this study was infants ≤ 2 year of age. Even though it was non-significant but this observation was supported by others [17,19]. They suggested that it may be attributed to immature immune system and parents anxiety seeking medical assistance in such young age. On the other hand an interesting finding by Martin and his colleagues found more frequent single infection in infant (birth–5 months) and more frequent mixed infection in (6-23 months) group. They explained that heightened immune response during a primary infection may discourage colonization by a second viral pathogen, leading to lowered prevalence of multiple viruses in young infants [20].

Respiratory virus	Type of infection			Total No. of isolated viruses (%)
	Single infection	Double infection N (%)	Triple infection	
	N (%)		N (%)	
RV	11 (20.4)	5 (9)	3 (5.6)	19 (35.2)
RSV	6 (11)	4 (7.4)	2 (3.7)	12 (22.2)
hMPV	7 (13)	2 (3.7)	1 (1.9)	10 (18.5)
AdV	5 (9.3)	2 (3.7)	2 (3.7)	9 (16.7)
HBoV	2 (3.7)	1 (1.9)	1 (1.9)	4 (7.4)
Total	31	14	9	54
FET=1.46 0.99 (NS)				

Table 4: Frequency of respiratory viruses according to the type of infections among 41 children.

Double and triple viral co-infection represented (17.1% and 7.3% respectively), which is comparable to other studies that ranged (12-20%) regarding double co-infection and (1.1-3.9%) for triple infection [19,21,22]. RV was the most frequently detected virus in single and mixed viral infection. This agreed with other studies (19,23). Other studies found RSV was the most common as a single virus [1] or as co-infection [5,6]. Al-Ayed and his colleagues mentioned that the most common viruses included in co-infection were RSV and RV [17]. It has been Reported the unique characteristic of RSV facilitates infection with a second respiratory virus [20,22]. Al-Ayed and others suggested that hRV in URTI's could serve as promotion factor which act synergistically to facilitate the pathogenesis of lower respiratory infections such as bronchiolitis [17,20,24].

Liu and his co-authors, suggested the hypothesis that the proportion of the specific pathogen, rather than the pathogen itself, is relevant for co-infections [18]. The discrepancy in proportion of viral agents may be due to differences in pathogen epidemiology, study populations, and/or the time the study was conducted due to seasonal variation. The leading combination in the current study was RV/AdV and RV/RSV, which was comparable to other studies [19,21,22]. Although RSV/hMpV combination was reported by some studies [17,25,26], this combination was absent in our study and other studies [19,27].

	Isolated viruses					Total n (%)	P
	HRV n (%)	RSV n (%)	hMPV n (%)	AdV n (%)	HBoV n (%)		
Age/month							
0-12 m	5 (9.3)	2 (3.7)	3 (5.6)	2 (3.7)	0 (0)	12 (22.2)	0.9 (NS)
13-24 m	7 (13)	5 (9.3)	2 (3.7) 1 (1.8)	4 (7.4)	3 (5.6)	21 (38.8)	
25-36 m	4 (7.4)	3(5.6)	2 (3.7)	1 (1.8)	0 (0)	9 (16.7)	
37m - 48 m	2 (3.7)	1 (1.8)	2 (3.7)	1 (1.8)	1 (1.8)	7 (13)	
49m-60m	1 (1.8)	1 (1.8)		1 (1.8)	0 (0)	5 (9.3)	
Gender							
Male	11 (20.4)	5 (9.3)	6 (11.1)	5 (9.3)	3 (5.6)	30 (55.6)	0.74 (NS)
Female	8 (14.8)	7 (13)	4 (7.4)	4 (7.4)	1 (1.8)	24 (44.4)	
Hospital unit							
Clinic	12 (22.2)	0 (0)	1 (1.8)	2 (3.7)	2 (3.7)	17 (31.5)	
PICU	0 (0)	6 (11.1)	3 (5.6)	2 (3.7)	0 (0)	11 (20.4)	0.004 (S)
Ward	7 (13)	6 (11.1)	6 (11.1)	5 (9.3)	2 (3.7)	26 (48.1)	
Clinical presentations							

URTIs	11 (20.4)	0 (0)	0 (0)	2 (3.7)	2 (3.7)	15 (27.8)	0.0001 (S)
Bronchitis	0 (0)	2 (3.7)	2 (3.7)	0 (0)	0 (0)	4 (7.4)	0.002 (S)
Bronchiolitis	3 (5.6)	6 (11.1)	4 (7.4)	2 (3.7)	1 (1.8)	16 (29.6)	0.000 (S)
Broncho pneumonia	4 (7.4)	4 (7.4)	2 (3.7)	4 (7.4)	0	14 (25.9)	0.03 (NS)
Pneumonia	1 (1.8)	0	2 (3.7)	1 (1.8)	1 (1.8)	5 (9.3)	0.000 (S)
Fisher's exact test (FET) was used							

Table 5: Distribution of respiratory viruses among positive 41 children in relation to different variables.

There is a debate about the significance of the newly identified HBoV as a cause of respiratory infection, which was isolated as a single virus and as a co-infection. Some studies reported that HBoV was isolated as a co-infection [28] and others suggested that the severity of symptoms of HBoV infection is related to viral load rather than the detection of virus [28,29].

Viruses	**URTIs**		**Bronchitis**		**Bronchiolitis**		**Broncho-pneumonia**		**Pneumonia**	
	Single	Mixed	Single	Mixed n (%)	Single n (%)	Mixed n (%)	Single	Mixed	Single n (%)	Mixed n (%)
	n (%)	n (%)	n (%)				n (%)	n (%)		
HRV	10 (18.5)	1 (1.8)	0 (0)	0 (0)	1 (1.8)	2 (3.7)	0 (0)	4 (7.4)	0 (0)	1 (1.8)
RSV	0 (0)	0 (0)	2 (3.7)	0 (0)	3 (5.6)	3 (5.6)	1 (1.8)	3 (5.6)	0 (0)	0 (0)
hMPV	0 (0)	0 (0)	2 (3.7)	0 (0)	3 (5.6)	1 (1.8)	1 (1.8)	1 (1.8)	1 (1.8)	1 (1.8)
AdV	1 (1.8)	1 (1.8)	0 (0)	0 (0)	1 (1.8)	1 (1.8)	2 (3.7)	2 (3.7)	1 (1.8)	0 (0)
HBoV	2 (3.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	1 (1.8)
P	0.074 (NS)		--------		0.702 (NS)		0.136 (NS)		0.154 (NS)	
FET was used										

Table 6: Correlation of isolated respiratory viruses (mono&co infection) with clinical outcome.

We assumed that mixed infection is more likely to cause more severe infection in the form of clinical outcome, hospitalization or ICU admission, but our study suggested non-significant association which goes in hand with others [4,20,30] . Even though some studies [5,19,21] showed significant association between severity of infection and the detection of mixed viral etiologies. Some studies went further to suggest that single infection resulted in more severe outcome [4,20].

Using PCR as a diagnostic tool is a point for discussion; study of Shafik and coworkers mentioned that viral shedding is mostly at the first three days of symptoms; Thus due to the high sensitivity of nucleic acid detection results must be interpreted with caution, particularly if the sample was taken late after symptom onset. So detection of viral co-infection by PCR can detect current and near past infections [1]. Quantitative assays can help resolve this problem. Nevertheless, Al-Ayed and his colleagues mentioned that multiplex RT-PCR is needed for epidemiological and virological data [17].

This study has limitations as it lacks bacterial studies, other viral etiologies and detection of viral loads. Also the short duration of the study made it unable to detect the seasonal variations of viral isolation. In spite of all this, it remains representative of viruses circulating in children under 5 years old in our patient groups. Several common or newly identified respiratory viruses were not assessed in this study, such as Picornaviruses, Coronaviruses, and newly discovered Polyomaviruses, so their contribution to respiratory disease etiology and rates of co-infection in this study remain unknown. These limitation can explain the number of cases which were symptomatic but negative by our PCR study panel.

In conclusion; this study reflected background view of respiratory viral etiology in children younger than 5 years old in Banha, Egypt. HRV was the most frequent viral pathogen either single or mixed infection. Mixed infection has no correlation with disease severity. Additionally, multiplex PCR is a useful tool for detecting mixed infection, but has to be interpreted with caution.

References

1. Shafik CF, Mohareb EW, Yassin AS, Amin MA, El Kholy A, et al. (2012) Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age. BMC Infectious Diseases 12: 350.
2. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, et al. (2007) Identification of a third human polyomavirus. J Virol 81: 4130–6. Retrieved 2008-01-18.
3. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, et al. (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoSPathog. 3 (5): e64. Retrieved 2008-01-18.

4. Chorazy ML, Lebeck MG, McCarthy TA, Richter SS, Torner JC, et al. (2013) Polymicrobial Acute Respiratory Infections in a Hospital-Based Pediatric Population. Pediatr Infect Dis J 32: 460–466.
5. Cui B, Zhang D, Pan H, Zhang F, Farrar J, et al. (2015) Viral etiology of acute respiratory infections among children and associated meteorological factors in southern China. BMC Infectious Diseases 15: 124.
6. Chu HY, Kuypers J, Renaud C, Wald A, Martin E, et al. (2013) Molecular epidemiology of respiratory syncytial virus transmission in childcare. J Clin Virol 57: 343-350.
7. Falsey AR (2008) Human metapneumovirus infection in adults. Pediatr Infect Dis J 27 (10 Suppl): S80–3.
8. Linder JE, Kraft DC, Mohamed Y, Lu Z, Heil L, et al. (2013) Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades. J Allergy Clin Immunol. 131: 69-77. e1-6.
9. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, et al. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA. 102: 12891-6.
10. Krakau M, Gerbershagen K, Frost U, Hinzke M, Brockmann M, et al. (2015) Case Report: Human Bocavirus Associated Pneumonia as Cause of Acute Injury, Cologne, Germany. Medicine (Baltimore) 94: e1587.
11. Tabain I, Ljubin-Sternak S, Cepin-Bogovic J, Markovinovic L, Knezovic I, et al. (2012) Adenovirus Respiratory Infections in Hospitalized Children: Clinical Findings in Relation to Species and Serotypes. Pediatr Infect Dis J 31: 680-4.
12. Wolfaardt M, Kiulia NM, Mwenda JM, Taylor MB (2011) Evidence of a recombinant wild-type human astrovirus strain from a Kenyan child with gastroenteritis. J Clin Microbiol 49: 728–731.
13. Kapusinszky B, Minor P, Delwart E (2012) Nearly Constant Shedding of Diverse Enteric Viruses by Two Healthy Infants. J Clin Microbiol 50: 3427–3434.
14. Coiras MT, Aguilar JC, Garci´a ML, Casas I, Pérez-Breña P (2004) Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 72: 484–495.
15. Peiris JSM, Tang WH, Chan KH, Khong PL, Guan Y, et al. (2003) Children with respiratory disease associated with metapneumovirus in Hong Kong. Emerg Infect Dis 9: 628–633.
16. Paton AW, Paton JC, Lawrence AJ, Goldwater PN, Harris RJ (1992) Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by reverse transcription and polymerase chain reaction amplification. J Clin Microbiol 30: 901-4.
17. Al-Ayed MS, Asaad AM, Qureshi MA, Ameen MS (2014) Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction. Saudi Med J 35: 11.
18. Liu J, Ai H, Xiong Y, Li F, Wen Z et al. (2015) Prevalence and Correlation of Infectious Agents in Hospitalized Children with Acute Respiratory Tract Infections in Central China. PLoS ONE 10: e0119170.
19. Essa S, Owayed A, Altawalah H, Khadadah M, Behbehani N, et al. (2015) Mixed Viral Infections Circulating in Hospitalized Patients with Respiratory Tract Infections in Kuwait. Hindawi Publishing Corporation. Advances in Virology 714062 8 pages.
20. Martin ET, Kuypers J, Wald A, Englund JA (2012) Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. Influenza Other Respi Viruses. 6: 71–7.
21. Harada Y, Kinoshita F, Yoshida LM, Minh le N, Suzuki M, et al. (2013) Does respiratory virus coinfection increases the clinical severity of acute respiratory infection among children infected with respiratory syncytial virus? The Pediatric Infectious Disease Journal 32: 441–445.
22. Kim JK, Jeon JS, Kim JW, Rheem I (2013) Epidemiology of respiratory viral infection using multiplex RT-PCR in Cheonan, Korea (2006–2010). J Microbiol Biotechnol 23: 267–273.
23. Suzuki A, Lupisan S, Furuse Y, Fuji N, Saito M, et al. (2012) Respiratory viruses from hospitalized children with severe pneumonia in the Philippines. BMC Infect Dis 12: 267–278.
24. Greenberg SB (2011) Update on rhinovirus and coronavirus infections. Semin Respir Crit Care Med 32: 433-446.
25. Canducci F, Debiaggi M, Sampaolo M, Marinozzi MC, Berrè S, et al. (2008) Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. J Med Virol 80: 716–723.
26. Xepapadaki P, Psarras S, Bossios A, Tsolia M, Gourgiotis D, et al. (2004) Human metapneumovirus as a causative agent of acute bronchiolitis in infants. J Clin Virol 30: 267–270.
27. Van Woensel JB, Bos AP, Lutter R, Rossen JW, Schuurman R (2006) Absence of human metapneumovirus coinfection in cases of severe respiratory syncytial virus infection. Pediatric Pulmonology 41: 872–874.
28. Christensen A, Nordbø SA, Krokstad S, Rognlien AG, Døllner H (2008) Human Bocavirus commonly involved in multiple viral airway infections. J Clin Virol 41: 34–37.
29. Söderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kemppainen K et al. (2009) Clinical assessment and improved diagnosis of Bocavirus-induced wheezing in children. Finland, Emerging Infectious Diseases 15: 1423-1430 .
30. De Paulis M, Gilio AE, Ferraro AA, Ferronato AE, do Sacramento PR et al. (2011) Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. J Pediatr (Rio J) 87: 307–313.