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CIRCULATING PATTERN OF RESPIRATORY VIRUSES, IN EGYPT, SEASON 2013-2014

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Respiratory viruses causes lower and upper respiratory tract infections; LRTIs and URTIs. These infections are from the predominant causes of infections, especially in children and infants.

***Objective**: This study, provides the evidence of using modern techniques as the multiplex PCR method, as a helpful tool in identifying the circulating pattern of respiratory viruses, in Egypt, thus this could help in identifying and inventing new treatment and vaccines.*

***Subjects and methods:** A number of 237 nasopharyngeal aspirates, were collected between December 2013 to November 2014, from 2 Egyptian fever hospitals, classified into 3 groups; group 1; 37 normal persons, group 2; 100 patients with LRTIs or pneumatic group and group 3; 100 patients with URTIs or common cold group. The results were then assayed using multiplex RT-PCR, in the Central Public Health Laboratories, CPHL, Ministry of Health, Cairo, Egypt.*

***Results:** Respiratory viruses, were detected in 152 cases, where the human rhinovirus was the most prominent; 15.6%, followed by the respiratory syncytial virus, 10.5%, metapneumovirus; 13%, influenza-A (Flu-A); 6.3%, adenovirus (AdV); 5.9%, influenza-B (Flu-B); 5.4%, parainfluenza-3 (PIV-3); 4% and a percentage of 0.42% infection for parainfluenza-1 (PIV-1), parainfluenza-2 (PIV-2) and human bocavirus (HBoV). No infections with parainfluenza-4, enterovirus nor the coronaviruses were detected. Both season and age of the patients, affect the detection rate of the analysis.*

INTRODUCTION

Upper and lower respiratory tract viral infections; URTIs and LRTIs, are among the most common illness in humans, where children and infants bear the major burden of infection, typically presenting 3-5 episodes of respiratory tract infections[1] or 5.06 episodes per child per year[2]. These infections have relation with significant patient morbidity and their related mortality[3], thus, these infections, represent the main reason of death in children younger than five years of age worldwide and this accounts for 1.9 million to 2.2 million death globally[4&5] and an estimate of 2 million children under five years according to the World Health Organization (WHO), die of pneumonia, yearly[6]. Acute respiratory tract infections, are the most common cause for admitting children to hospital globally[7] and

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febrile episodes in infants younger than three months of age[8]. Their infections are mainly of viral etiology, while the bacterial etiology represents only 10% of all URTIs with the subsequent 90% of infections caused by respiratory viruses[9]. Despite this viral etiological origin, antibiotics are often mistakenly prescribed in the treatment of URTIs, exacerbating ineffective treatments, antibiotic abuse and also bacterial resistance[10].

Chronic co-morbidities or accompanied risk factors especially lung diseases like asthma or chronic obstructive pulmonary disease (COPD), increase the load of respiratory tract infections, especially in young ages or people with decreased immunity[11&12]. The viruses primarily associated with URTIs commonly include rhinoviruses; HRV, enteroviruses; HEV, adenoviruses; AdV, parainfluenza viruses PIV; types 1, 2, 3 and 4, influenza viruses A and B (Flu-A and Flu-B), respiratory syncytial viruses; RSV and human coronaviruses[8,13&14].

From the beginning of the 21st century, nearly every year of the first decade, there was a newly discovered virus, which cause can respiratory diseases to human. In 2001, human metapneumovirus; hMPV, was discovered in the Netherlands, from family *Paramyxoviridae*[15]. In the two following years, 2002-2003, a new human coronaviruses, known as Severe Acute Respiratory Syndrome coronavirus; SARS-CoV was discovered, in China[16]. Then in 2004, another human coronavirus known as NL63; HCoV-NL63, was discovered, this time in Amsterdam[17]. Later in the following year, 2005, two more respiratory viruses were discovered, one from the *Parvoviridae* known as the human bocavirus; HBoV[18&19] and the other was from the *Coronaviridae* known as HKU1; HCoV-HKU1, in Hong Kong[20]. The last discovered virus from the *Coronaviridae* was in 2012 and known as the Middle East Respiratory Syndrome coronavirus; MERS-CoV, and it was discovered in Saudi Arabia[21]. This discoveries introduced 6 new respiratory viruses, from the year 2001 till 2012, from which four of them were from the *Coronaviridae*, one from the *Paramyxoviridae* and one from the *Parvoviridae*.

Clinical differentiation between respiratory viruses is somehow difficult due to the similarity in their symptoms, either local as cough and rhinitis or systemic, as fever and headache. Also the influenza, in which its diagnosis has clinical bases, it is also inaccurate[22]. The clinical manifestations or by symptoms differentiation can be applied only for young children, who can't discuss their symptoms, or how they feel. Knowing the cause or the etiology of the respiratory infection are of great need for establishing paediatric emergency department at hospitals or clinics, to propose the needed anti-influenza therapy[23], and also to prevent nosocomial infection and to decrease the infection control methods, to get the most benefits of viral detection techniques, from lowering chest X-rays examination, other laboratory investigations and avoiding antibiotics overuse and better prognosis of the clinical routes of treatments[24].

High sensitivity, short time in getting results and good availability, are the main advantages that makes polymerase chain reaction; PCR, the basic method in diagnosing respiratory viruses, in comparison to other conventional methods such as tissue culture[25]. The Multiplex reverse transcription; RT-PCR methods, allows detection of several viruses at the same time[26], to overcome the any disadvantage of being performed in a laboratory or does not provide information timely for clinical decisions at the emergency department.

Point of care (POC) tests, are designed to be available directly at the place of patient care. This place must be accessible to staff who are not trained in laboratory, and provide quick results, to affect the clinical decision making[27]. These tests are frequently used for Influenza type A and B and also for the RSV. To carry out these tests, there is a need of working persons at the proper time and this is why the emergency department, practically use this test. This study will detail these newly discovered and emerging respiratory viruses and also will focus on influenza virus host interaction and possible treatments.

The aim of this study is to provide, a helpful tool; multiplex PCR method, for identification of the circulating pattern of respiratory viruses, in Egypt, thus help in improving and inventing the required vaccines, providing them with the most important strains

according to the discovered results and investigating new treatment in accordance to the needs of our community, to respond to the identified viruses and thus decrease the health burden, hospitalization periods, and accompanied common co-morbidities and also provide each group of patients with a known infected virus with the proper handling methods, from hospitalization, ventilation, follow-up, …. etc.

MATERIAL AND METHOD

Two hundred and thirty seven patients were admitted to Imbaba and Menia fever hospitals, in Egypt, for one whole year, from December 2013 to November 2014, were included in our study. These patients were either with hypothetical diagnosis of an acute URTIs and LRTIs, or were undergoing surgical therapy. The diagnosis by the URTIs and LRTIs, was determined by doctors and physicians in these hospitals. The patients were then classified into three groups; Group 1 control; included 37 normal persons whom were underlying surgical therapy, Group 2; including 100 patients with LRTI or pneumatic group, and Group 3; including 100 patients with URTI or ILI; Influenza-like illness, or common cold symptoms group. The study was approved by the health research ethics committee and all tests were carried out in central public health laboratories (CPHL), Cairo, Egypt.

Specimens

Nasopharyngeal aspirate (NPA) samples were collected, in sterile vials; Nasopharyngeal Specimen Collection Flocked Swab, from each enrolled patient. According to the manufactures, this swabs were prepared using technology called nylon fibre and under specific condition of using fields of electro-static power, and were provided by vertical flocking, which were finely fixed and were paired with the virus transport media tube; VTM, which provides safety in storage and transportation. For the samples to be taken from the patients, their head were tilted backward for a degree of about 70°, then horizontally tip were placed into one of the nose ditch, till the postal nasopharynx, then the stick rotated twice to allow the tip suck up sufficient aimed sample, then the swab was slowly pulled out and then put down instantly into the sterile VTM tube. The proper timing of collecting samples are between day one of illness and within two weeks. The VTM tubes containing the specimens, were kept on ice and handled during 2 hrs of collection[28]. All samples were anonymously labelled with specific study numbers and accessioning numbers, then stored at -80°C until they are assayed. Clinical management and outcome data were collected.

Group 1: Control group

Thirty seven normal persons, who were underlying surgical therapy in Imbaba and Menia fever hospitals, with no respiratory tract symptoms, were our selected control group. Patients confirmed with respiratory infections within the 10 days before NPA collection, were excluded from the study.

Group 2: LRTI (Pneumatic group)

One hundred patients with LRTIs include pneumonia, defined according to WHO definitions, bronchitis and croup or severe pneumonia if there is drawing lower chest[29]. Symptoms of LRTIs usually involve runny nose, or its congestion, sore throat, and may be accompanied by cough, fever or lethargy and this symptoms changes by changing degree of infection from acute to severe infection.

Group 3: URTIs or ILI or common cold group

One hundred patients with UTRIs with symptoms vary from sinuses in the paranasal area, to systemic illness in pharynx and larynx. To recognize them from other URTI, after their exposure to respiratory viruses, with mean of 2 days, they have no cough, examined free from pharyngitis caused by *Streptococcus* for two weeks before the examination, have nasal congestion with or without sore throat, they have one weeks usually fever accompanied with acute sinusitis and conjunctivitis. The exclusion criteria was patients confirmed with LRTIs within 10 days prior to collection of NPA[30&31].

Methods

Multiplex RT-PCR technique was used for the detection of the respiratory[32]. This process

was carried out at the division of medical virology, at the Central public health laboratories.

Thawing the samples at a temperature of 22°C. On the automated QIA symphony platform (Qiagen, Germany) the extraction of the total nucleic acids was done utilizing the system's DSP virus/ Pathogen Mini Kit. The application protocol was carried out according to the instructions of QIA symphony, employing free 200V5 virus as the complex cell.

The 16 respiratory viruses, assayed in this research were amplified using the Multiplex RT-PCR and shown in table 1. The instrument used was the real-time PCR machine Bio-Rad CFX96TM (Bio-Rad, Hercules, California, US) and according to (Seegene, South Korea) as a manufacture, the Anyplex II RV 16 assay was used. For this test, 100 copies/reaction were our lower limit of viral detection.

Table 1: Viruses tested with the Anyplex II detections System A set- B set.

PIV4	PIV1	PIV2	PIV3
AdV	Flu A	Flu B	HRVC
hMPV	229E	NL63	RSV
HBoV	HRV-B	HEV	HRV-A

Multiplex RT-PCR amplification for 16 commonly respiratory viruses causing infections; 14 different RNA viruses; Flu-A/B, PIV-1/2/3, RSV-A/B, HRV-A/B/C, CoV-229E, NL69, HEV, hMPV and two different DNA viruses; HBov and AdV, performed on the BioRad CFX96TM RT- PCR instrument, using the Anyplex II RV 16 assay (Seegene, South Korea) as per the manufacturer's recommendations. The reported lower limit of detection of this assay is 100 copies per reaction.
Respiratory virus detection reaction, are divided into two panels A and B, where each detected nine targets (8 viruses and one internal control).

Reverse transcription

The extracted RNAs synthesize the cDNAs using cDNA Synthesis Premix (Seegene Inc. Soul, South Korea). All 237 samples were tested using Anyplex II RV16 (Seegene Inc., Soul, South Korea). Respiratory virus detection reaction, was divided into two panels A and B, where each detected nine targets (8 viruses and one internal control), 14 different RNA viruses; Flu-A/B, PIV-1/2/3, RSV-A/B, HRV-A/B/C, CoV-229E, NL69, HEV, hMPV and two different DNA viruses; HBoV and AdV. The technologies of DPO™ and TOCE™ are used by the multiplex PCR technique to identify and differentiate the 16 respiratory viruses. The RT-PCR instrument ends the process with melting curve analyses. Anyplex II RV16 detects: Flu-A, Flu-B, PIV-1/2/3, RSV-A/B, AdV, HRV A/B/C, CoV 229E, NL69, HEV, hMPV and HBoV.

The viruses were identified on the CFX96 instrument, by the amplicon specific melting temperature and one of the four fluorophores labelling. Seegene Viewer software analysed the results of this amplification.

Reverse transcription procedure

- 8 µl of the extracted nucleic acid, 0.2 µg random hexamers, and 3 µl nuclease-free water were added to a sterile, nuclease-free tube on ice.
- The tube was incubated at 80°C for 3 mins in a Bio-Rad CFX96TM real-time PCR instrument (Bio-Rad, Hercules, California, US), chilled on ice for 2 mins and spun down briefly in a Spectrafuge Mini Centrifuge (Lab net International, New Jersey, USA).
- For a 20 µl final reaction volume, 4 µl 5X reaction buffer plus 1 µl Ribolock™ RNase inhibitor (20 u/µl) plus 2 µl 10mM dNTP mix plus 1 µl Revert Aid™ M-MuLV Reverse Transcriptase (200 u/µl) were added to the tube.
- The tube was incubated twice. The first time was at 37°C for 90 min. The second time was at 94°C for only 2 mins in a Bio-Rad CFX96TM real-time PCR instrument (Bio-Rad, Hercules, California, US).
- The tube was chilled on ice for 2 mins and spun down briefly in a Spectrafuge Mini Centrifuge before storage at –20°C until amplification process.

Amplification

All samples were amplified at the same time, each one in three separate reactions (Set A, B, and C). The content of each reaction is listed in table 2 and 3, showing the Anyplex® RV16 PCR reagent volume and the cycling and amplification protocol for multiplex real time PCR reaction, respectively.

RESULTS AND DISCUSSION

Results

Study groups

152 viruses in the total 237 (group 1; control, group 2; LTRI and group 3; URTI) tested specimens were detected with a percent of 64.1% of the total samples studied. Single infections were 103 while multiple infections were 49. The highest infection was for the HRV, which was detected in 37 samples and divided into 26 single infection and 11 co-infection, followed by the RSV with 36 positive cases, followed by the rest of the respiratory viruses, except for the PIV-4, HEV and the human coronaviruses which were not detected in all the tested specimens.

Table 2: Anyplex® RV16 PCR reagent volume.

Reagent	Content	Volume (µl)
5X RV16 PM	— Primer pairs for pathogens in each set (A, B or C)	5
	— Primer pair for internal control	
	— Template for internal control	
RNase free water	8-methoxypsoralen (8-MOP) to prevent carry-over contamination	2
4X Anyplex PCR master mix (with UDG)	— DNA polymerase	5
	— Buffer containing dNTPs	
	— $MgCl_2$ and stabilisers	
Nucleic acid sample		8
Total volume		20

Table 3: Cycling and amplification protocol for multiplex Real Time PCR reaction.

Segment	No. of cycles	Temperature	Duration
1	1	50°C	4 min
2	1	95°C	15 min
3	30	95°C	0.5 min
4		60°C	1.0 min
5		72°C	0.5 min
6	1	55°C	0.5 min
7	1	Melting curve 55°C -85°C (5s/0.5°C)	
8	10	95°C	0.5 min
9		60°C	1.0 min
10		72°C	0.5 min
11	1	55°C	0.5 min
12	1	Melting curve 55°C -85°C (5s/0.5°C)	
13	10	95°C	0.5 min
14		60°C	1.0 min
15		72°C	0.5 min
16	1	55°C	0.5 min
17	1	Melting curve 55°C -85°C (5s/0.5°C)	

No respiratory viruses were detected in 85 specimens, giving a ratio of 35.9% negative cases. To identify and provide the required collective data, about the most circulating strains of respiratory viruses either causing LRTIs, URTIs or even asymptomatic persons and thus propose the required strains to be included in vaccines and suggest investigating new drugs for the discovered strains, in Egypt, the numbers and percentages of the detected viruses are shown in (Table 4).

Group 1: Control group

Eight samples, with positive respiratory viruses, were detected in control group in the asymptomatic patients so, we decided to include these sample to the study groups, as it is important to indicate the total prevalence rate of respiratory viruses, in Egypt.

Group 2: LRTI (Pneumatic group)

The numbers and percentages of the detected viruses in group 2 are shown in (Table 5).

Table 4: Viruses detected in single and multiple infections in all three groups; Group 1, 2 and 3, by multiplex RT-PCR.

Total groups	HRV	RSV	hMPV	Flu-A	AdV	Flu-B	PIV-3	PIV-1	PIV-2	HBoV
	N-37, 15.6%	N-36, 15.1%	N-25, 10.5%	N-15, 6.3%	N-14, 5.9%	N-13, 5.4%	N-9, 4%	N-1, 0.42%	N-1, 0.42%	N-1, 0.42%
HRV	26	4	3	1	2	1	0	0	0	0
RSV	4	26	1	1	1	2	1	0	0	0
hMPV	3	1	18	1	2	0	0	0	0	0
Flu-A	1	1	1	11	0	1	0	0	0	0
AdV	2	1	2	0	7	1	1	0	0	0
Flu-B	1	2	0	1	1	7	1	0	0	0
PIV-3	0	1	0	0	1	1	6	0	0	0
PIV-1	0	0	0	0	0	0	0	1	0	0
PIV-2	0	0	0	0	0	0	0	0	1	0
HBoV	0	0	0	0	0	0	0	0	0	1

N: Represent number of positive samples for each virus.
%: Represent the proportion of positive samples of each virus, from the total number of samples tested; 237 samples.
Triple infections which were detected in the study: HRV/RSV/AdV > 2.
HRV/RSV/FLU-A → 3 HRV/RSV/FLU-B → 2 RSV/AdV/PIV-3 → 1 HRV/ hMPV /Adv →1

Table 5: Viruses detected in single and multiple infections in group 2; pneumatic group.

Total groups	HRV	RSV	hMPV	Flu-A	AdV	Flu-B	PIV-3	PIV- 1	PIV- 2	HBoV
	N-17, 17.0%	N-15, 14%	N-13, 13%	N-3, 3%	N-1, 1%	N-1, 1%	N-7, 7%	N-0, 0%	N-1, 1%	N-1, 1%
HRV	14	2	1	0	0	0	0	0	0	0
RSV	2	13	0	0	0	0	0	0	0	0
hMPV	1	0	12	0	0	0	0	0	0	0
Flu-A	0	0	0	3	0	0	0	0	0	0
AdV	0	0	0	0	1	0	0	0	0	0
Flu-B	0	0	0	0	0	1	0	0	0	0
PIV-3	0	0	0	0	0	0	7	0	0	0
PIV-1	0	0	0	0	0	0	0	0	0	0
PIV-2	0	0	0	0	0	0	0	0	1	0
HBoV	0	0	0	0	0	0	0	0	0	1

N: Represent number of positive samples for each virus.
%: Represent the proportion of positive samples of each virus, from the total number of samples tested of this group (100 patients).

17 cases infected with HRV (14 single infection and 3 mixed infection) were found in the pneumatic group, 15 cases infected with RSV (13 single and 2 mixed infection with HRV), 13 infection with hMPV (only 1 mixed infection with the HRV), 7 cases in PIV-3, 3 cases in Flu-A, 1 case only in PIV-2, Flu-B, HBoV and AdV PIV-3. No infections with PIV-1, PIV-4, HEV nor the human coronaviruses were detected in the tested specimens for this group.

Group 3: URTIs or ILI or common cold group

The numbers and percentages of the detected viruses in group 3 are shown in (Table 6). 47 mixed infection and 5 triple infections were found in the URTIs or the common cold group. The highest number of infection was detected in RSV with 17 infected cases (10 single and 7 mixed infections), followed by the HRV with 15 infected cases (11 single and 4 mixed cases), 13 cases in both AdV and Flu-B, 12 cases in both hMPV and Flu-A, 2 cases in PIV-3 and only 1 case in PIV-1. No infections with PIV-2, PIV-4, HBoV, HEV nor the human coronaviruses were detected in the tested specimens for this group.

Demographic characteristics, clinical picture, gender, admission department and outcome of study samples, including HRV positive samples and subtypes

HRV infections were detected in 37 patients representing 15.6%. HRV-A was the most widespread species, where 19 out of 37 were HRV-A infected, representing 51.35%, followed by HRV-C, with 14 out of 37, representing 37.8% then the HRV-B species with 4 out of 37, representing 10.8%. From the total number of all the HRV species' infections, there were 32 patients only with available age information. The age range for was between 0.01 and 81 years. The mean age was 20.9 with standard deviation 23.9 and median equals to 7. Demographic analysis indicated that 20.6% of the total population was less than two years of age. The overall rate of the age group <2 years was greater than the other age groups, especially for HRV-A and HRV-C infections. HRV was detected more in males (63%) especially in HRV-A and HRV-C infections also, showing that gender might be considered also as a risk factor for HRV infections. Dyspnea and abnormal breathing sounds, are major symptoms of HRV infection and which were found in all the HRV-B cases. For the total study population about the hospitalized percent was 61.8% and this was at the ICU, from the total HRV cases, the hospitalized patients in the ICU were 58.9%. (Table 7).

Table 6: Viruses detected in single and multiple infections in group 3, URTIs or ILI or common cold group.

Total groups	HRV N-15, 15%	RSV N-17, 17%	hMPV N-12, 12%	Flu-A N-12, 12%	AdV N-13, 13%	Flu-B N-13, 13%	PIV-3 N-2, 2%	PIV-1 N-1, 1%	PIV-2 N-0, 0%	HBoV N-0, 0%
HRV	11	1	2	1	2	1	0	0	0	0
RSV	1	10	1	1	1	2	1	0	0	0
hMPV	0	1	6	1	2	0	0	0	0	0
Flu-A	1	1	1	8	0	1	0	0	0	0
AdV	1	1	2	0	6	1	1	0	0	0
Flu-B	1	2	0	1	1	7	0	0	0	0
PIV-3	0	1	0	0	1	1	2	0	0	0
PIV-1	0	0	0	0	0	0	0	1	0	0
PIV-2	0	0	0	0	0	0	0	0	0	0
HBoV	0	0	0	0	0	0	0	0	0	0

N: Represent number of positive samples for each virus.

%: Represent the proportion of positive samples of each virus, from the total number of samples tested of this group (100 patients).

Table 7: Demographic characteristics, clinical picture, gender, admission department and outcome of study samples, including HRV positive samples and subtypes.

Age groups (years)	Total Study population (%)	Total HRV	HRV-A	HRV-B	HRV-C
< 2	20.6%[a]	26.3%[b]	29.8%	5.3%	26.9%
2-	13.8%	17.5%	15.5%	10.5%	25%
5-	5.7%	8.8%	9.5%	5.3%	7.7%
10-	5.2%	8.8%	9.5%	0%	11.5%
20-	9.6%	8.1%	4.8%	31.6%	5.8%
30-	8.5%	4.4%	3.6%	0%	7.7%
40-	8.1%	6.3%	4.8%	15.8%	3.8%
50-	13.3%	10.6%	11.9%	10.5%	9.6%
≥ 60	15.1%	9.4%	10.7%	21.1%	1.9%
Total: N (%)	237 (100%)	37 (100%)	19 (100%)	4 (100%)	19 (100%)
Gender					
Male	59%[c]	63%[d]	66%[d]	45%	64%[e]
Female	41%	37%	34%	55%	36%
Total: N (%)	237 (100%)	37 (100%)	19 (100%)	4 (100%)	14 (100%)
Clinical Picture					
Dyspnea[e]	90.5%	88.3%	80.4%	100%	96.2%
Abnormal Breathsound[e]	79.4%	83.6%	78.3%	100%	84.9%
Tachypnea	58.5%	99 (57.9%)	48.9%	75%	67.9%
Sputum	63.4%	93 (54.4%)	54.3%	60%	52.8%
Sore throat	49.6%	79 (46.2%)	47.8%	55%	39.6%
Chest Pain	4.5%	8 (4.7%)	4.3%	5%	5.7%
Hemoptysis	2.5%	5 (2.9%)	2.2%	0%	5.7%
Total: N (%)	237 (100%)	37 (100%)	19 (100%)	4 (100%)	14 (100%)
Admission Department					
ICU	147 (61.8%[f])	22 (58.9%[g])	11 (59.3%)	3 (75%)	8 (57%)
Pediatrics	23.9%	28.6%	26.4%	5%	43.1%
Medical Ward	14.3%	12.5%	14.3%	25%	2%
Total: N (%)	237 (100%)	37 (100%)	19 (100%)	4 (100%)	14 (100%)
Outcome					
Discharge	74.3%	71.7%	58 (66.7%)	12 (66.7%)	40 (81.6%)
Transfer	20%	22%	22 (25.3%)	5 (27.8%)	7 (14.3%)
Death	4.2%	16.2%	4 (10.8%)	1 (5.6%)	1 (2.7%)
Unknown	1.6%	2.5%	3 (3.4%)	0 (0%)	1 (2%)
Total: N (%)	237 (100%)	37 (100%)	19 (100%)	4 (100%)	14 (100%)

N: Represent number of positive samples for each virus

%: Represent the proportion of positive samples of each virus from the total number of samples tested of this group.

Clinical data collected from questionnaire.

[a]For study population: 20.6 % of total populations are less than two years of age.

[b]For total HRV: Children under two years of age are infected with 26.3 % of total HRV infections.

[c] Males represent 59% of the total study populations.

[d]About two third of total HRV infections are among males; 63%, similarly, HRV-A and HRV-C while only 45% of HRV-B are among males.

[e]Dyspnea and abnormal breathing sounds are major symptoms of HRV infection which reaches up to 100% in HRV-B infection.

[f]For study population: 61.8% were admitted to the ICU.

[g]For total HRV cases: 58.9% were admitted to the ICU.

HRV infection, was found in the pneumatic group and was represented all year around especially, between January to April and then between August to December, where HRV-A infection was the most predominant type, between January to April then between August and September with the exception of December where HRV-C was the prevalent type. HRV-B was mostly detected between July and December. HRV monthly distribution percentages is shown in figure 1.

Correlation between human rhinovirus species and pneumatic group (LRTIs)

In the common cold or URTIs group, there were significant correlations between HRV-A and HRV-C and pneumonia, with R values of 0.08 (p= 0.037, p< 0.05) and 0.20 (p< 0.001), respectively (Table 8). No significant correlation was identified for HRV-A, -B, and -C infections between the pneumatic group and the control group (p> 0.05).

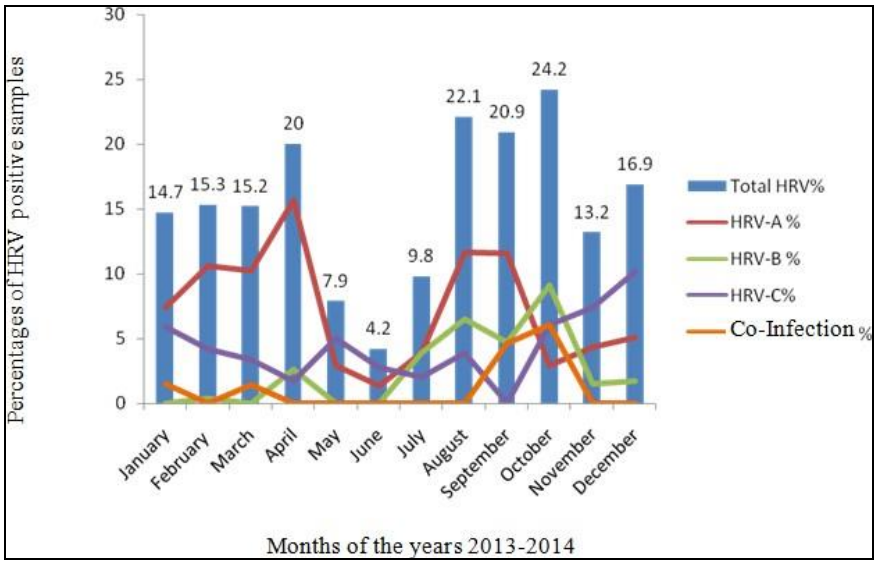

Fig. 1: Percentages of HRV types detected by month.

Total HRV percentage/month is indicated over the corresponding bar.

Table 8: Correlation between human rhinovirus species and pneumatic group.

Groups compared with pneumonia group	HRV species P	Spearman's R	*P*
Control group (*n*= 37)	HRV-A (*n*= 2)	0.031	0.622
	HRV-B (*n*= 1)	0.101	0.12
	HRV-C (*n*= 2)	0.095	0.161
Common cold UTRIs group (*n*= 100)	HRV-A (*n*= 7)	0.082	0.037
	HRV-B (*n*= 1)	0.019	0.98
	HRV-C (*n*= 7)	0.202	< 0.01

In the common cold or URTIs group, there were significant correlations between HRV-A and HRV-C and pneumonia, with R values of 0.08 (p= 0.037, p< 0.05) and 0.20 (p< 0.001), respectively (Table 8). No significant correlation was identified for HRV A, B, and C infections between the pneumonia group and control group (p> 0.05).

Discussion

In the present study, the Seegene Inc., Soul, invented technology, known as the DPO-TOCE, was used to describe the operating and specific detection of sixteen respiratory viruses, at the same time[33]. We compare our results to many other published studies in a number of different countries, the RSV was the most common virus in patients complaining from LRTIs[14], followed by adenovirus[34] and influenza viruses H3N2 and H1N1[35] in 2012 and was published in western Europe, in the same year same results were published also in Africa in Ghana[36] and in an earlier year in 2011, was published in Egypt[37]. This was in contrast to our findings, where the most recurrent virus was the HRV are (15.6%), followed by the RSV, the hMPV and then the influenza viruses. But our results were similar to other studies which judge the HRV as an major cause of respiratory tract infection[38&39] and which induce wheezing and aid in worsening of asthma[40]. Single infection with HRV was found in 10 (4.21%) samples and co-infection was found in 26 (10.9%) samples. HRV represents the chief cause of severe acute respiratory tract infections; SARI, for all age population and represents 24.3% of cases in which a virus was detected.

Males and young children were the majority of the patients whom were admitted to hospital, as found in other studies[41]. One week was the median period of staying in hospitals; this is compatible with other published papers in other countries and continents as Eastern Europe, Ghana in Africa and in Asia in both Saudi Arabia and the United Arab Emirates, respectively[35,36,42&43].

Highest co-infection rate was found with RSV in 4 cases (10.8%). This finding is concurrent with other reports[44]. Following the RSV in number of co-infection, was the hMPV with 3 co-infection cases (8.9%), in which two cases from them (66%) were paediatrics admitted to the ICU. Same results was published by other studies[45]. Low co-infection rate with influenza viruses, was noticed in our study, where only two cases (7.7%) were detected. This is consistent with other reports, stating that the increase in the pandemic influenza virus, could be obstructed because of infection with HRV[46&47].

In our study, HRV-A and HRV-C were found in 18 (50.5%) and 12 (31%) patients representing 12 and 8 types of each, respectively. However, we detected HRV-B from only 4 (10.8%) patients representing 4 types of HRV-B. So, HRV-C was found with a frequency less than that of HRV-A, but greater than that of HRV-B and this also occurred in other studied populations[48].

A large number of circulating types of rhinovirus was discovered till now[49], within our study we discovered the HRV-C, in the subject population from 7 sites all over Egypt. These results are concurrent with Wisdom *et al.*[50].

In our study, the more severe illness were associated with HRV-A infection than the HRV-C, where it represented four out of each six fatal cases. Our results were opposite to other published papers as in Longtin *et al.*[51], in which that HRV-C species was suggested as the more severe and pathogenic species, than the other HRV species[48,52&53].

Difference in the age distributions, was not our aim from our study but the clinical and epidemiological information collected from patients, provided important findings in this filed. The age profile for HRV-A and HRV-C infection were similar but HRV-B was found more in older age groups (>21 years old). In our study group, HRV-A and HRV-C were over examplificated significantly in males than in females where in males, HRV-A and HRV-C infections were found approximately two third that in females. This finding is in contrast with what was found by others[50].

Larger sample number, over a longer period of time and across a larger number of governorates, is recommended for further studies.

Oxygen supplement was required for influenza patients with any type of respiratory tract infection, than the non-influenza patients, as published in other studies[7]. All over the year, UTRI and LRTIs took place in our study and in other countries, as in the United Arab Emirates[43], but these infections were higher common in autumn and winter in India, and extent in July and August during the season of rain not all over the year[54]. In our study, co-morbidity with heart diseases was frequent for LRTIs patients who stayed in hospitals. This is in contrast to other studies that stated that the heart, the nervous system and the

neuromuscular diseases cardiac, are not considered as risk factors for long stay in hospitals, among positive influenza patients[55&56]. This may be due to geographical differences, differences in pathogens or differences methods of diagnosis instead of the design of the study or the methods used in them.

Lower age mean was discovered in the RSV cases than the non-RSV cases, with 64% of them younger than 6 months old. In many studies, the young age was reported as a risk factor for RSV infection[57]. Some studies associated this to the decrease in the cellular immunity[58], and the decrease in maternal antibodies acquired from their mothers, attributed with decrease in the humoral immune response magnitude to RSV, in patients aged <3 months[59]. Cough and difficulty in breathing was chronic and happened in almost all LRTIs patients, in our study. We observed higher respiratory rates, in RSV patients, especially in patients younger than one year in old; 80% of the RSV cases; this was also observed by other studies[60].

Determining the geographical distribution of RSV infection, is a useful tool for guess the probability of epidemics and design control and preventive methods, especially for the high risk groups. The highest hospitalization rate of RSV infection was demonstrated in our study (3 times greater than other etiologies, $p< 0.001$), which is consistent with other studies[61] and the highest one require oxygen supplementation. This cases also have higher fatality rate, but this may be due to the small number of deaths, that may make a clinically significant comparison. Prematurity, diseases in lung and heart, are also among the familiar risk factors attributed to RSV infection.

We need a fast, precise and cheap diagnostic tool, for diagnosing respiratory viruses, to control and prevent the annual influenza and other circulatory respiratory viruses.

Two studies cited the incidence of viral respiratory viruses in Egypt. The first study was published 2016, and was from June 2009 till December 2013, on 6113 patients, in Damanhur, showed a 67% incidence rate in patients less than one year and 19% incidence rate in patients over 65 years of age[62].

The second study, which was published 2019 and was done from the period 2010-2014, at Cairo University Hospital, showed an incidence rate viral respiratory viruses in Egypt of 33.5% in hospitalized adult and paediatric Egyptian patients with SARI[63].

Conclusion

The circulating pattern of respiratory viruses epidemiology, demonstrated that the HRV were the most common one followed by the RSV, the hMPV and then the influenza viruses. HRV-A might be linked to more severe illness than HRV-C, with nearly similar age profile, in contrast to HRV-B which was found more in older age groups (>21 years old). Thus when providing HRV vaccine, the main strain to be included in the vaccine, especially for children, should be HRV-A, also, new drugs must be investigated, prescribed and licensed for HRV-A.

While in infections with RSV, most cases were severe, with high hospitalization rate, with significantly lower age mean and had well-known medical risk factors, as prematurity, chronic lung disease and congenital heart disease, so it must be also included in children's vaccine.

It should be noticed that most positive patients for influenza virus, generally required oxygen therapy more often than other patients. Heart examination must be included in follow-up for hospitalized LRTIs patients, as the cardiac diseases were the most common co-morbidity. The study of the local epidemiology of respiratory viruses, is essential yearly, for predicting epidemics and planning for preventive measures, especially for the high risk groups.

Abbreviations

RT-PCR	Reverse transcription Polymerase chain reaction.
LRTIs	Lower respiratory tract infection.
URTIs	Upper respiratory tract infection.
ILI	Influenza- like illness.
WHO	World Health Organization
NPA	Nasopharyngeal aspirates.
VTM	Viral transport medium.
HEV	Human enterovirus.
HRV	Human rhinovirus.
RSV	Human respiratory syncytial virus.
hMPV	Human metapneumovirus.

Flu-A	Influenza-A.
Flu-B	Influenza-B.
Adv	Adenovirus.
PIV-1	Parainfluenza-1.
PIV-2	Parainfluenza-2.
PIV-3	Parainfluenza-3.
PIV-4	Parainfluenza-4.
HBoV	Human bocavirus.
ICU	Intensive care unit.

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النمط المنتشر لفيروسات الجهاز التنفسي ، في مصر ، موسم ٢٠١٣-٢٠١٤

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نبذة مختصرة: الالتهابات الفيروسية في الجهاز التنفسي السفلي LRTIs والعلوي ، URTIs من أكثر الأمراض شيوعًا بين البشر ، وخاصة الأطفال والرضع.

أهداف البحث: تقدم هذه الدراسة الدليل على استخدام طريقة تفاعل البوليميراز المتسلسل المتعدد PCR كأداة مفيدة في تصنيف النمط المنتشر لفيروسات الجهاز التنفسي لتحديد وابتكار علاجات ولقاحات جديدة.

التجربه: تم جمع ٢٣٧ مسحا أنفيًا بلعوميًا بين الفتره من ديسمبر ٢٠١٣ الى نوفمبر ٢٠١٤ ، وتم تقسيمها الى ثلاث مجموعات ؛ مجموعة ١ ؛ تتكون من ٣٧ شخصًا عاديًا ، المجموعة ٢ ؛ تتكون من ١٠٠ مريض يعانون من عدوى الجهاز التنفسي السفلي LRTIs ، والمجموعة ٣ ؛ تتكون من ١٠٠ مريض يعانون من عدوى الجهاز التنفسى العلوى URTIs ، ثم يعايروا باستخدام طريقة Multiplex RT-PCR.

النتائج: تم الكشف عن فيروسات الجهاز التنفسي في ١٥٢ حالة ، حيث كان فيروس الأنفلونزا HRV; Rhinovirus هو الأبرز ، يليه الفيروس المخلوي التنفسي Respiratory syncytial virus; RSV ، الفيروس التالي لالتهاب الرئة البشري Human metapneumovirus; hMPV ، والإنفلونزا أ / ب FLU-A/B ، ونظير الإنفلونزا ١-٣ Parainfluenza-1-3 ، والفيروس الغدي البشرى adenovirus والفيروس البوكا Bocavirus. ولم يتم الكشف عن فيروسات كورونا Coronavirus، ولا فيروس نظير الأنفلونزا ٤ Parinfluenza-4 ، ولا الفيروس المعوي Enterovirus. وقد أظهرت النتائج أيضا ان كل من موسم وعمر المرضى يؤثر على معدل اكتشاف نتائج التحاليل.