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Respiratory Viruses and Atypical Bacteria Co-Infection in Children with Acute Respiratory Infection

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Abstract

BACKGROUND: Acute respiratory infections (ARI) are one of the prevalent pediatric diseases. Coinfections of respiratory viruses and atypical bacterial respiratory pathogens are common.

AIM: This study aimed to determine the prevalence of co-infection between respiratory pathogens including viruses, bacteria and atypical bacteria in a sample of Egyptian children presenting with symptoms of acute respiratory tract infection.

METHODS: This one-year prospective cohort study conducted in Abo El Rish Pediatric Hospital, Cairo University over one year included children presenting with symptoms of acute respiratory infection. Enrolled children were subjected to nasopharyngeal swabs or throat swabs and then processed to detect viral, bacterial and atypical bacterial causative agents by culture), retrotranscription polymerase, Monoplex polymerase chain reaction (PCR) and Multiplex PCR.

RESULTS: Viral etiological agents were detected in 20 cases (20.8%), while 76 patients (79.2%) had no definite viral aetiology. The most abundant virus detected was Rhinovirus in 36 (27.3%), followed by 21 (15.9%) were positive for RSV, 12 (9.1%) were positive for HMPV, 6 (4.5%) were positive for adenovirus and 3 (2.3%) were positive for influenza B. For Atypical bacterial causes Mycoplasma were positive for 9 (6.8%) cases and one case was positive for Bordetella parapertussis. Viral and atypical bacteria Co infection were detected in 14 (10.6%) of cases.

CONCLUSION: These results suggest that coinfection with bacteria or atypical bacteria in children with acute respiratory tract infection is common and this co-infection can induce serious illness. The multiplex reverse-transcriptase polymerase chain reaction should become an essential tool for epidemiological studies and can fill the gap between clinical presentation and definitive diagnosis.

Introduction

Acute respiratory infections (ARI) are one of the prevalent pediatric diseases all over the world. In children below five years [1] [2] [3], acute respiratory infections are responsible for 18–33% of all deaths. Unfortunately, more than half of this mortality is recorded among developing countries as low social level and malnutrition double the burden [2] [3] [4].

Acute respiratory infections can be caused by pathogens like bacteria, viruses, fungal and atypical bacteria include Streptococcus pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, and respiratory viruses (influenza A and B, adenovirus, respiratory syncytial virus and parainfluenza) [5] [6].

Although viruses are usually the main responsible for most of both upper and lower acute respiratory infections (ARIs) [7], but studies documented that patients may be infected with both bacterial and viral pathogens, making it difficult to identify the clinical characteristics that allow the physician to differentiate viral disease from bacterial disease in the early course of the disease [8].

Recently, many new emerging respiratory viruses have been identified including human coronavirus (HCoV), NL63 [9] and human bocavirus

(HBoV) [10]. Moreover, various emerging respiratory viruses led to epidemics and pandemic (H1N1) virus infection and H5N1 [11].

Atypical pathogens Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila are considered important agents are causing mild, moderate, or even severe acute respiratory tract infections (ARTIs) in children worldwide [12].

This study aimed to determine the prevalence of co-infection distribution between respiratory pathogens including viruses and atypical bacteria among a well cohort of febrile children presenting by acute respiratory tract infections symptoms.

Material and Methods

This one-year prospective cohort study conducted in Abo El Rish Pediatric Hospital, Cairo University over one year from the first of June 2016 to 30th of June 2017 on children presenting with symptoms of acute respiratory infection.

Ethical statement

The study followed the regulations of the medical ethical committee of the Cairo University and the medical ethical committee of the National Research Centre and. Signed informed consents were collected from legal child guardian before participation after explaining the purpose of the study.

Any child presented with Influenza-like illness (ILI) symptoms the chest clinic Abo El Rish Pediatric Hospital, Cairo University was considered eligible. ILI was defined according to CDC criteria as having measured fever > 38˚C and cough and/or a sore throat [13].

Exclusion criteria

Any child receiving immunosuppressive therapy started antibiotic therapy, children with chronic lung diseases and patients who were unwilling to participate in this study were excluded from the study.

Data collection

Data were collected by a self-designed questionnaire include demographic data, risk factors (smoking, passive smoking and contact with birds). Throat washes and nasal swabs were collected from each child as appropriate.

Laboratory analysis

For Routine Bacterial and fungal culture

A throat wash was taken from each child when appropriate, in the case of a young infant throat swab was taken instead.

Specimens then were cultured on blood agar, chocolate agar, MacConkey agar and Sabarauds dextrose agar (Oxoid Co. England). Isolates were identified by conventional methods such as culture characteristics and biochemical reactions.

For Viral PCR

Nasopharyngeal swabs were taken from each participant by measuring the distance between the ear lobule and the ala nasi by the NP swab and divided by 2, and the swab marked at this distance to ensure the insertion of the swab in the proper site. This flexible, sterile tip flocked with nylon fibre swab applicator was inserted into the nostril and back to the nasopharynx and left in place for a few seconds. It was then slowly withdrawn with a rotating motion. The swab was placed in a 15 mL centrifuge tube labelled with the unique patient ID and containing 2 mL viral transport media (VTM: consisting of a sterile solution of bovine albumin fraction V, HEPES buffer, penicillin and streptomycin in HANK's balanced salt solution). The applicator stick was then cut off.

The received swabs inside the 15 ml tube were agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip, and then swab was removed from the tube and discarded using a forceps. The VTM was immediately placed in a freezer (-70°C) until tested with PCR for the presence of respiratory viruses. If samples are positive for Influenza A, then further testing was performed by PCR to determine if the virus is a seasonal influenza variant or swine H1N1 2009 virus.

PCR testing for Respiratory viruses

Viral Nucleic Acid Extraction: Viral Nucleic acid extraction was done after centrifugation of the sample for 10-20 min and 200ul from the sediment taking as starting material using the QIAamp® Viral RNA Mini cat number 52904 according to the manufacturer's instructions. Elution was done with 60ul buffer AVE, and reelution step was added to increase the nucleic acid concentration.

CDNe was done using 15ul from the extracted RNA, for only the testing of the following RNA viruses (Rhinovirus, Influenza, RSV and HMPV). For Adenovirus conventional PCR testing was done directly from the extracted nucleic acid (DNA). The kit used for complementary DNA synthesis was Sensifast cDNA (cat# BIO-65054).

PCR: Conventional PCR was done by 3 different reactions for each sample as follows for the following viruses:

- Monoplex PCR for Rhinovirus testing using primers sequence, concentrations and conditions as [14];
- Monoplex PCR for Adenovirus testing using primers sequence, concentrations and conditions as [14];
- Multiplex PCR for InfA, InfB, HMPV and RSV testing from the following work [15] (Table 1).

Table 1: Primer list for detection of Respiratory viruses by different PCR types

Virus-tested	Type of PCR	Primers			Final Primers Conc	Master Mix used	Annealing Temp	Ref
Rhino	Monoplex	EVP4	5'-CTACTTTGGTGTCCGTGTT-3'		0.2µM	One Taq Hot Start Quick-Load 2X Master Mix (M0488S)	55°C	[2]
		OL68-1	5'-GGTAAYTTCCACCACCANCC-3'					
Adenovirus	Monoplex	AdnU-S'2	5'-TTCCCCATGGCNCACAAYAC-3'		0.2µM	One Taq Hot Start Quick-Load 2X Master Mix (M0488S)	59°C	[2]
		AdnU	5'-TGCCKRCTCATRGGCTGRAAGTT-3'					
InFA and B, RSV HMPV	Multiplex	RSV	vrs P1	GGA ACA AGT TGT TGAGGT TTA TGA ATA TGC	0.3µM	Qiagen multiplex MM	55°C	[1]
			vrs P2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT				
		InFA	mia 1	CAG AGA CTT GAA GAT GTC TTT GCT GG	0.3µM			
			mia 2	GCT CTG TCC ATG TTA TTT G				
		InfB	mib 1	AAA ATT ACA CTG TTG GTT CGG TG	0.3µM			
			mib 2	AGC GTT CCT AGT TTT ACT TG				
		hMPV	hmpv 1	CCC TTT GTT TCA GGC CAA	0.3µM			
			hmpv 2	GCA GCT TCA ACA GTA GCT G				

Statistical method

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data were summarised using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage)

Results

The study was conducted from first June 2016 to June 2017. 132 children presented by ILI were enrolled in the study selected from Pediatric Hospital Cairo University. 57 (43.2%) were males, and 75 (56.8%) were females.

The age of patients ranged from 1month-10 years (120 months) with a mean age of 2.95 ± 2.46 years (Table 2), this study was designed to estimate the prevalence of respiratory pathogens in 132 children presented with ARI. Overall, samples from 114 individuals (86.3%) were found to be positive for at least one pathogen, and 18 of them were positive for two or more pathogens. Rhinovirus was the most commonly detected agent, followed by *Streptococcus pneumoniae*.

Table 2: Demographic data of the studied children

Age (months)		
Mean ±SD	29.34 ± 28.29	
Range	1-120	
	Frequency	Percentage
Gender		
Female	57	43.2%
Male	75	56.8%
Exposure to smoking		
Yes	63	62.8%
No	49	37.1%
Exposure to birds		
Yes	61	46.2%
No	71	53.8%

Viral etiological agents were detected in 78 cases (59.09%), while 54 patients (40.9%) had no definite viral aetiology. The most abundant virus detected was Rhinovirus in 36 (27.3%), followed by 21 (15.9%) were positive for RSV, 12 (9.1%) were positive for HMPV, 6 (4.5%) were positive for adenovirus and 3 (2.3%) were positive for influenza B (Table 3).

Table 3: Etiological agents detected in the studied children

Etiological agents	Frequency	Percentage
Viral agents		
Rhinovirus	36	27.3
RSV	21	15.9
HMPV	12	9.1
Inf A	0	0
Inf B	3	2.3
Adenovirus	6	4.5
Bacterial agents		
Klebsiella	17	12.9
Enterobacter	11	8.3
Pseudomonas spp.	11	8.3
Acinetobacter	9	6.8
Klebsiella and enterobacter	1	0.8
Atypical bacteria agents		
Chlamydophila pneumoniae	0	0
Bordetella parapertussis	1	0.8
Bordetella pertussis	0	0
Legionella pneumophila	0	0
Mycoplasma pneumoniae	9	6.8

Bacterial agents were detected in 49 cases (37.1%), Klebsiella was detected in 17 (12.9%) child followed by Enterobacter in 11 (8.3%), Pseudomonas spp. 11 (8.3%), Acinetobacter 9 (6.8%) and Klebsiella and Enterobacter co-infection in one child (0.8%).

For Atypical bacterial causes, Mycoplasma was positive for 9 (6.8%) cases, and one case was positive for Bordetella parapertussis (Table 3).

Table 4: Co-infection distribution among studied cases

Co-infection	No
Streptococcus pneumoniae, mycoplasm, Rhinovirus and HMPV	3
Streptococcus pneumoniae, and Rhinovirus	1
Streptococcus pneumoniae, Rhinovirus and HMPV	2
Streptococcus pneumoniae and Rhinovirus	6
Rhinovirus and HMPV	3
Mycoplasm and RSV	3

Viral and atypical bacteria Co infection were detected in 14 cases illustrated in Table 4.

Discussion

Acute respiratory disease present about 75% of all acute morbidities in developed countries, and most of the cases (about 80%) are viral [16]. On average, five to eight respiratory viruses are detected in pediatric patients every year [17] [18]. These viruses are well-known causes of acute respiratory tract 6+03infections (ARTIs), which are a major source of morbidity and mortality in infants and young children [19].

The PCR technology development allowed a wide range of etiological viral agents to be detected with more sensitivity and specificity [20].

Several recent studies have analysed the epidemiologic pattern of respiratory viral infections; Rhinovirus is usually the most common single pathogen found in ARI surveillances samples with prevalence range 24. -50% followed by RSV 22-25% and influenza viruses 7.2-8% either by detection in nasal washes or nasopharyngeal swabs [21] [22] [23], which comes in accordance with an Egyptian study by Amin et al., 2012 RSV virus was detected with a high predominance (51.9%) in Egyptian asthmatic children with acute respiratory tract infection [24].

In the current study, the most abundant virus detected was Rhinovirus in 36 positive samples (27.3%), followed by RSV in 21 (15.9%) positive samples. Although it is non-statistically significant, all the positive cases are distributed all over different age group.

Human metapneumovirus (HMPV) is a recently identified Paramyxovirus first isolated from hospitalised children with acute respiratory tract infections (ARTI) in 2001 [25] Since then the hMPV reported prevalence ranged between 1.5 to 17.5% [26].

In the current study positive samples for hMPV was 12 (9.1%), This ratio may be considered lower than similar studies [26] [27] [28] and that can be explained by that child included in our study were enrolled from the patient presenting by ILI symptoms and the hMPV is more common in hospitalized children with pneumonia .

The dual viral infection or the multiple viral isolates occurrence was reported by some studies [29] [30] on the contrary; other studies failed to detect more than one viral agent [24]. In our study, we reported 3 cases of rhino and hMPV dual viral infection.

Atypical bacteria as Mycoplasma pneumoniae, Bordetella parapertussis and Chlamydia pneumoniae are common respiratory pathogens in children 5 years of age and older [31]. Infection may be preceded by an upper respiratory infection. Of the 132-child presented by ILI in our study 9 (6.8%) children were positive for Mycoplasma pneumoniae, and one case (0.8%) was positive to Bordetella parapertussis.

Coinfections with respiratory viruses and bacterial respiratory pathogens are common, often via synergistic interactions between the viruses like influenza virus, the bacteria, and the human host, However, the interaction between viruses and atypical bacteria still unexplained [32].

This study aimed to demonstrate the rate of co-infection between viral and bacterial agent. Although all the children enrolled in this study had the similar initial clinical presentation but the causative agent varied from single or multiple and bacterial or viral .

Viral and bacterial Co-infection was detected in 18 (22.7%) of children in the current study, of them 6 children were positive to Rhinovirus and another agent either bacterial (Streptococcus pneumoniae), atypical bacteria (Mycoplasma), viral (HMPV) or multiple causative agents.

The dual infection or multiple causative agent infections had been studied evidently in recent researches as multiplex PCR enabled laboratorians to detect a panel of viruses simultaneously while reducing hands-on time and with greater analytical sensitivity. There are several multiplex assays evaluated for detection of respiratory viruses [33].

In a study on respiratory pathogens in hospitalized children, of 1281 children, 449 (35%) had an acute respiratory tract infection caused by at least one of the organisms studied; there were 29 cases of dual infection [34]. In another study Serology with paired samples and PCR on nasopharyngeal aspirates and throat cultures were used to identify bacteria and viruses and mixed viral/bacterial pathogens in 26 patients (20.5%) The main etiological agents were adenovirus, respiratory syncytial virus (RSV), Mycoplasma pneumoniae, Streptococcus pyogenes and Chlamydia pneumoniae [35].

These results suggest that coinfection with bacteria or atypical bacteria in children with acute respiratory tract infection is common and this co infection can induce serious illness.

Unfortunately, most of respiratory tract infection are still treated empirically [36] with antibiotic in spite that most of the causative agents are viral on the other hand Most of pneumonia caused by atypical bacteria usually occurs after infection of the upper respiratory tract [32].

Certainly, the early management of respiratory tract infection is essential to ensure good prognosis and avoid complications. The diagnosis based on clinical finding alone is no more suitable the need of non-traditional diagnostic test now is becoming a must as molecular methods such as multiplex Real-Time PCR (RT-PCR) facilitate

identifying many causative agents which in turn reduce the excessive use antibiotics has caused rising bacterial resistance.

The multiplex reverse-transcriptase polymerase chain reaction should become an important tool for epidemiological studies and can fill the gap between clinical presentation and definitive diagnosis.

In conclusion, co-infection with multiple pathogens with the predominance of viruses is emerging. The Large-scale multicentric studies are recommended to address the epidemiological gap, and hence a new management strategy can be developed.

Limitation of the study

Small sample size and a low number of co-infection cases preclude any robust statistical analysis.

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