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Incidence and Clinical Course of Respiratory Viral Coinfections in Children Aged 0–59 Months

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Abstract

Clinical data available on coinfections are contradictory concerning both the number of viruses involved and the severity of the condition. A total of 114 patients aged 0–59 months with symptoms of respiratory tract infection were enrolled into the study. Nasal and pharyngeal swabs were tested using the PCR method for the following 12 viruses: influenza A, influenza B, respiratory syncytial virus A (RSV A), respiratory syncytial virus B (RSV B), adenovirus, metapneumovirus, coronavirus 229E/NL63 (hCoV229), coronavirus OC43 (hCoVOC43), parainfluenza virus 1 (PIV-1), parainfluenza virus 2 (PIV-2), parainfluenza virus 3 (PIV-3), and rhinovirus A/B. Coinfections were detected in nine (8 %) patients. Five of the coinfections were related to influenza A (H3N2) virus associated with the following other, single or combined, respiratory viruses: influenza B in one case, hCoV229 in two cases, hCoV229, RSV A, and PIV-2 in one case, and PIV-1, PIV-2, RSV A, RSV B, and adenovirus in one case. The other four coinfections were caused by: adenovirus and hCoVOC43, adenovirus, and rhinovirus, RSV A and PIV-1, influenza B, and RSV B. We did not observe any significant differences in the clinical course of infections caused either by a single or multiple viral factors.

Keywords

Children • Coinfection • Etiology • Pathogenesis of infection • Respiratory tract disease • Virus

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1 Introduction

Respiratory viral infections are a major source of morbidity and mortality in childhood. Children with respiratory infections are generally treated as outpatients and the etiology of their disease is usually not investigated. In case of hospitalization, the diagnostic techniques employed are often not sensitive enough (Jin et al. 2007). It is estimated that 50–85 % of pediatric acute respiratory tract infections are of viral origin. Experience and understanding of the role of viral coinfections in respiratory tract infections have grown in recent years due to the introduction of molecular techniques (Brunstein et al. 2008; Mahony et al. 2007). At present, clinical data available on confection are variable, sometimes contradictory, in terms of both the number of viruses involved and the severity of the condition. These discrepancies may be due to such factors as geographical region and detection methods (Kumar 2009). The detection of respiratory viruses in children using molecular methods can be challenging. The reason for this is that virus-virus coinfections and mixed viral-bacterial infections occur in 15–30 % of cases and viruses can be detected in 25–45 % of children in the absence of respiratory symptoms (Frobert et al. 2011; Peng et al. 2009; Raymond et al. 2009). Although, the effects of viral coinfections have been described and analyzed in the literature, the number of such studies is limited, especially in Central Eastern Europe, including Poland. The aim of this study was to analyze the incidence and clinical course of respiratory tract infections caused by more than one viral etiological factor among children aged 0–59 months.

2 Material and Methods

Ethical approval was obtained from the Medical University of Warsaw, Poland. Data and specimens were obtained from patients aged 0–59 months with respiratory tract infection symptoms: fever $> 38°C$, sore throat or cough lasting less than 96 h. The exclusion criteria were the following: symptoms lasting longer than 96 h, age > 59 months, ongoing antibiotic therapy, and the child's guardian refusal to take swabs. A total of 114 patients were enrolled into the study: 52 children hospitalized in the General Pediatric Ward and 62 children requiring ambulatory care; all with acute respiratory tract infections. Guardians of all the children enrolled were provided with written information regarding the study's aims and methods, and written consent was obtained from all the guardians. Two swabs were taken from the patients: one nasal and one pharyngeal. Viscose swabs were used to collect specimens that were stored for less than 24 h at a temperature of 2–8 °C and then transported to the National Influenza Center at the National Institute of Public Health – National Institute of Hygiene in Warsaw, Poland, where specimens from patients, stored at -80 °C, were tested by RT-PCR using a RV12 ACE Detection Kit (Seegene, Seoul, South Korea) for the detection of the following respiratory viruses: influenza A virus, influenza B virus, human respiratory syncytial virus A (RSV A), human respiratory syncytial virus B (RSV B), human adenovirus, human metapneumovirus, human coronavirus 229E/NL63 (hCoV229), human coronavirus OC43 (hCoVOC43), human parainfluenza virus 1 (PIV-1), human parainfluenza virus 2 (PIV-2), human parainfluenza virus 3 (PIV-3), and human rhinovirus A/B. A random hexamer primer for cDNA synthesis was used with the First Strand cDNA Synthesis Kit (Fermentas, York, UK). Each cDNA preparation was subject to the RV12 PCR procedure according to the manufacturer's instructions (Seegene, Seoul, South Korea). Afterwards, amplicons were detected using gel electrophoresis. The subtyping of influenza viruses was carried out using conventional multiplex RT-PCR (Influenza A/B OneStep Typing Set; Seegene, Seoul, South Korea). A panel of Seeplex RT-PCR assays was used to detect influenza A, influenza B, and the three subtypes of influenza A (H1, H3, and H1N1 2009).

A descriptive analysis of patients with coinfections was carried out, including age, gender and symptoms. A comparison of symptoms of infections caused by a single viral agent and

those caused by more than one agent was performed. The statistical analysis was conducted with a medical statistical calculator available at www.medcalc3000.com

3 Results

Nine patients (8 %) were infected by more than one viral agent; 64 (56 %) patients were infected by a single virus; 41 (36 %) samples were negative. Single etiological agents were as follows: influenza A (29 cases, 25 %), RSV B (18 cases, 16 %), RSV A (5 cases, 4 %), adenovirus (4 cases, 4 %), HCoVOC43 (3 cases, 3 %), hCoV229 (2 cases, 2 %), PIV-1 (1 case, 1 %) and PIV-2 (1 case, 1 %). Five cases caused by influenza A (H3N2) virus were associated with coinfections with other respiratory viruses, such as influenza B (1 case), hCoV229 (2 cases), hCoV229, RSV A, and PIV-2 combined (1 case), and PIV-1, PIV-2, RSV A, RSV B, and adenovirus combined (1 case). Figure 1 presents an image of PCR test results for a multiple coinfection with six respiratory viruses.

The remaining four were double infections caused by adenovirus and hCoVOC43, adenovirus and rhinovirus, RSV A and PIV-1, and influenza B and RSV B. The demographical and clinical characteristics of patients with coinfections are presented in Table 1. The incidence of viral coinfections was similar among hospitalized and non-hospitalized children with symptoms of respiratory tract infection (7.6 % *vs.* 8 %, respectively). Symptoms and clinical course of the disease caused by either single or multiple agents were similar; no significant differences were observed (Table 2).

4 Discussion

In the present study, the prevalence of viral coinfections among young children with acute respiratory tract infection was on the low side of 8 %. In the literature, coinfection rates appreciably vary depending on the study. Peng et al. (2009) found multiple etiological agents in 36 % of hospitalized children; coinfections occurred predominantly among children aged 3–6 years and the most common pathogens were influenza A, influenza B, and PIV-1. Renois et al. (2010) detected 17 % of multiple infections among patients with acute respiratory tract infections; they were combinations of influenza A/H1N1v virus with CoV, human bocavirus (HBoV), RSV or human rhinoviruses (HRVs). Bonzel et al. (2008) diagnosed viral coinfections in 16 % of samples, with RSV and hBoV being the most common combination. In that study, viral coinfection was found in 17 % of children with bronchitis and in 23 % of those with bronchiolitis, while in

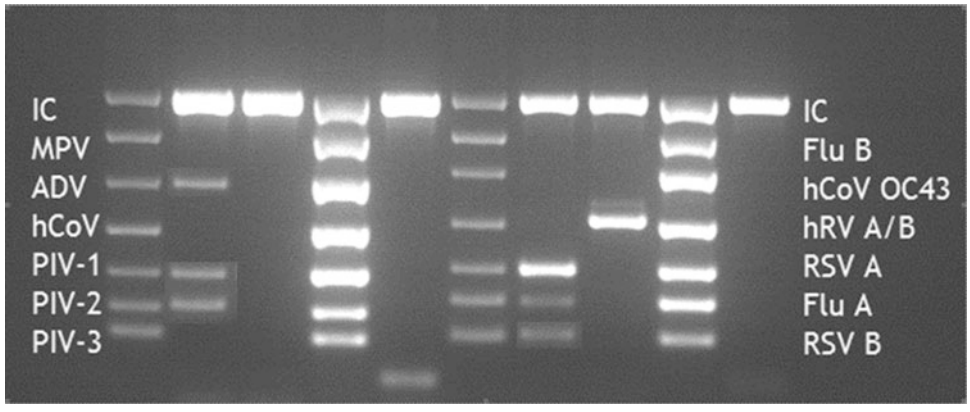

Fig. 1 PCR results for simultaneous infections with six respiratory viruses: influenza A (Flu A), parainfluenza 2 (PIV-1), parainfluenza 1 (PIV-2), respiratory syncytial virus A (RSV A), respiratory syncytial virus B (RSV B), and adenovirus (ADV)

Table 1 Characteristics of patients with coinfections

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
	Influenza A	Influenza A	Influenza A	Influenza A	Influenza A	Adenovirus	Adenovirus	RSVA	Influenza B
				Parainfluenza 2	Parainfluenza 2				
				hCoV229	Parainfluenza 1				
					RSV A				
					RSV B				
Etiology	Influenza B	hCoV229	hCoV229	RSV A	Adenovirus	hCoOC43	Rhinovirus	Parainfluenza 1	RSVB
Age (months)	11	12	16	5	3	4	3	1	2
Gender	M	M	M	F	M	F	F	M	F
Ambulatory care	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Hospital care	No	No	No	No	No	Yes	Yes	Yes	Yes
Sore throat	Yes	No	Yes	No	Yes	No	No	No	No
Sneezing/coryza	No	No	No	Yes	Yes	No	No	No	
Cough	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Dyspnea	No	Yes	No	No	No	No	No	No	No
Shortness of breath	No	No	Yes	No	No	No	No	No	No
Wheezing	No	No	No	No	No	No	No	No	No
Cyanosis	No	No	No	No	No	No	No	No	No
Tachycardia	No	No	Yes	No	No	No	No	No	No
Tachypnea	No	No	No	Yes	No	No	No	No	No
Abnormal pulmonary breathing sounds	Yes	No	Yes	No	Yes	No	No	No	No
Chills/convulsions	No	No	No	No	No	No	No	No	No
Vomiting/diarrhea	No	No	No	No	No	Yes	No	No	No

hCoV229 human coronavirus 229E/NL63, *hCoVOC43* human coronavirus OC43, *RSV A* respiratory syncytial virus A, *RSV B* respiratory syncytial virus B

Table 2 Clinical course and symptoms of infections caused by multiple and single agents

	Multiple viral agents (9 cases)	Single viral agents (64 cases)	p-value, OR, 95 % CI
Ambulatory care	5	30	$p > 0.05$, OR 1.41, 95 % CI 0.37–5.35
Hospital care	4	32	$p > 0.05$, OR 0.80, 95 % CI 0.21–3.03
Sneezing/coryza	2	31	$p > 0.05$, OR 0.30 95 % CI 0.06–1.41
Cough	6	50	$p > 0.05$, OR 0.56, 95 % CI 0.13–2.30
Sore throat	3	42	$p > 0.05$, OR 0.26, 95 % CI 0.06–1.06
Dyspnea	1	8	$p > 0.05$, OR 0.87, 95 % CI 0.12–6.32
Shortness of breath	1	10	$p > 0.05$, OR 0.67, 95 % CI 0.10–4.76
Wheezing	0	3	$p > 0.05$, OR 0.02, 95 % CI 0.02–9.74
Cyanosis	0	2	$p > 0.05$, OR 0.02, 95 % CI 0.01–14.77
Tachycardia	1	6	$p > 0.05$, OR 0.86, 95 % CI 0.17–9.02
Tachypnea	1	7	$p > 0.05$, OR 1.01, 95 % CI 0.14–7.41
Abnormal breathing sounds	3	7	$p > 0.05$, OR 2.32, 95 % CI 0.23–8.43
Chills/convulsions	0	8	$p > 0.05$, OR 0.02, 95 % CI 0.01–3.25
Vomiting/diarrhea	1	8	$p > 0.05$, OR 0.87, 95 % CI 0.12–6.32

OR odds ratio, *CI* confidence intervals

patients with pneumonia 33 % were positive for 2 or more viral pathogens. In the present study, the majority of coinfections were associated with influenza A infection and all of them occurred in children younger than 24 months.

We failed to not observe any significant differences in the clinical course of infections caused by either a single or multiple viral factors. Our data are in agreement with other studies that have reported no clinical differences between patients with respiratory infections caused by a single or multiple agents detected in nasopharyngeal aspirates from children (Martin et al. 2012; Nascimiento et al. 2010). Camargo et al. (2012) found coinfections with influenza A H1N1 in 22 % of patients with no greater morbidity or mortality. Suryadevara et al. (2011) diagnosed 28 % of multipathogen viral infections among febrile children younger than 24 months with acute respiratory infections. The most common viruses detected were RSV and rhinovirus/enterovirus. There were no differences in the severity of disease when comparing patients infected with one or multiple pathogens. However, there are some studies that show that coinfection may be a risk factor for poor evolution. Aberle et al. (2005) reported that airway obstruction was more severe when RSV contributed to coinfection and the hospital stay was lengthened when rhinovirus was involved. In another study, Greensill et al. 2003 showed that when coinfection consisted of RSV and metapneumovirus, evolution was worse. Further, Richard et al. (2008), who studied the relationship of coinfection with the need for admission to the pediatric intensive care unit (PICU), concluded

that the presence of two or more viruses increased the likelihood of admission. However, in another study conducted among patients admitted to the PICU, Ghani et al. (2012) found that bacterial coinfection was only associated with a longer hospital stay, with no increase in mortality. Calvo et al. (2008) reported multiple viral infections in hospitalized infants with respiratory tract disease in about 17 % cases and these coinfections were linked to higher fever, longer hospital stays, and a more frequent use of antibiotics compared with the cases of single RSV infections. It is worth noting that the detection of more than one virus may be due to the presence of viral fragments persisting for up to 5–6 weeks after the onset of symptoms, which may actually be irrelevant for the current clinical course. Further studies are required to confirm or exclude associations between co-detected respiratory viruses and to demonstrate the underlying mechanisms of such associations which may lead to cooperation or competition between viruses. Another important issue that remains unclear is the long-term effect that such coinfections may have on the development of chronic lung disease.

A limitation of the present study was a relatively small number of cases evaluated compared with the usually high numbers seen in pediatric practice. Another limitation was the lack of inclusion of bacterial coinfections. Predisposing factors for synchronous coinfection were not detected in our study, although there are some other data pointing to such a possibility. For instance, a high rate of codetection of HBoV with HAdV was reported and a prolonged shedding of the HBoV may potentially contribute to this phenomenon (Jartii et al. 2011). On the other hand, an advantage of the present study was that we described viral coinfections among young immunocompetent children, while other authors often report multiple infections among immunosupressed patients (Stefanska et al. 2013).

To conclude, viral respiratory coinfections do occur in young immunocompetent young children, but such infections do not seem to play an important role in the clinical presentation of acute respiratory infections in this age group. Studies involving larger numbers of patients and using improved quantitative detection techniques are needed to define the exact role of viral coinfections in the course of respiratory disease.

Conflicts of Interests The authors declare no conflicts of interest in relation to this article.

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