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High incidence of viral co-infections and atypical bacterial detection in acute respiratory infections among hospitalized children in the Central Department of Paraguay, 2010—2011

Griffiths and colleagues, in this Journal, recently highlighted the nature and consequences of microbial co-infections. Their results suggest differences between co-infected patients and those with single infections, with co-infection having serious health effects.[1] The objective of this study was to determine the prevalence of viral and atypical bacterial pathogens as single or as co-infections in hospitalized children with acute respiratory infections (ARIs) in Paraguay. ARIs with 3.5 million deaths annually, account for 20% of all deaths in pre-school children worldwide, with 90% of these deaths due to pneumonia. Risk factors for severe ARI include malnutrition, low birth weight, passive smoking, no breastfeeding, low socio-economic status, overcrowding, immunodeficiency and HIV infection, and consequently, the greatest morbidity associated with ARIs occurs in the developing world.[2] The most frequently implicated viruses consistently shown to predominate among hospitalized children with ARIs are respiratory syncytial virus (RSV), human rhinovirus (hRV), human metapneumovirus (hMPV), and parainfluenza viruses (PIV1-2-3-4). Other commonly implicated pathogens are influenza A and B viruses (FluA and Flu B), adenovirus (AdV), human coronavirus (hCoV) and enterovirus (EV), and human bocavirus (hBoV).[3—7] The expansion in the number of circulating pathogens, combined with an increased capacity to simultaneously test for multiple organisms through the use of recent advances in molecular detection,[5,8] has highlighted the potential impact of co-infections, when more than one pathogen from a single sample has been identified.

Between May 2010 and October 2011, we recruited 76 children (54 males) less than 2 years of age (median age 5.9 months, IQR 1—24), hospitalized at the Hospital General Pediátrico Niños de Acosta Ñu (HGP), a public pediatric teaching hospital. This Hospital provides medical care mainly to low-income families residing in the Central Department, which has a population of around 2 million (~25% of Paraguayan population), including approximately 210,000 children under 5 years of age, and a density of 800 inhabitants/km^2.

Children with symptoms of severe acute respiratory infection (upper and/or lower respiratory tract manifestations of ARI) with an onset of illness less than 7 days before hospitalization without pneumonia were included in this study. Bronchiolitis was diagnosed in children, whose upper respiratory symptoms preceded lower respiratory symptoms of wheeze, tachypnea, and other signs of respiratory distress.

DNA and RNA extracted from 200 μL of nasopharyngeal aspirate (NAs), using the silica powder method,[9] were tested for the presence of 18 viral and 5 bacterial respiratory pathogens in six multiplex reverse-transcription PCR reactions by Real Time PCR (qPCR) using the kit Fast-Track Respiratory pathogens plus (Fast-Track Diagnostics, Luxembourg), and analyzed on an ABI 7500 thermocycler (Applied Biosystems, USA).

Ninety-eight viruses were identified in 88.2% (67/76) of cases and 77.6% of the patients carried at least one pathogenic bacterium in NA. The most commonly identified viruses were rhinovirus (30% of cases); parainfluenza viruses (25.0%) with PIV3 representing the majority 8/19 (42.0%), followed by PIV1 6/19 (31.6%); coronaviruses were found in 18 samples (23.7%) with OC43 being the most common, i.e. 13/18 (72.2%) among CoVs positive samples; RSV (18.4%), AdV (9.2%), hBoV (13.2%) and hMPV (5.3%) (Table 1). Bacterial colonization was also detected, that include *Streptococcus pneumoniae* (Spn) (68.4%), *Staphylococcus aureus* (Sau) (23.7%), *Mycoplasma pneumoniae* (Mpp) (5.3%) and *Haemophilus influenzae B* (HiB) (3.8%) (Table 1). Influenza A, B and H1N1, *parechovirus* (PV), and *Chlamydia pneumoniae* were not detected in these 76 cases.

Single viral infections accounted for 59.2% (45/76) of cases. Viral co-infection was detected in 20 patients (26.3%), and viral/bacterial pathogens in 54 (71%). Among viral co-infections, dual infections were identified in 19.7% of cases (15/76), triple infections in 3.9% (3/76), while 4 and 5 viral co-infections were detected in two cases.

Bronchiolitis 53/76 (69.7%), EVW/asthma 15/76 (19.7%) and URTI 8/76 (10.5%) comprised the majority of discharge diagnoses. More than two days of hospitalization were required in 53% patients (median time 4.5 days, IQR 1—19). Out of 53 cases with bronchiolitis, 46 (87%) were children under 6 months of age, in whom rhinovirus, CoV OC43 and RSV represented the majority of infections. Up to 70% of children with severe bronchiolitis had at least one bacterium in their NA; and 40 children under 6 months of age (75.5%) carried *Streptococcus*

Table 1 Viral and bacterial co-infections by infectious agent.

Virus	Single viral infection	Co-infection	Total	%
Rhinovirus	9	14	23	30.3%
Human coronavirus (OC43, NL63, 229E, HKU)	8	10	18	23.7%
Parainfluenza (1—4)	14	4	18	23.7%
Respiratory syncytial virus	8	6	14	18.4%
Bocavirus	4	6	10	13.2%
Adenovirus	0	7	7	9.2%
Human metapneumovirus	2	2	4	5.3%
Enterovirus	0	3	3	3.9%
Parechovirus	0	0	0	0.0%
Influenza A	0	0	0	0.0%
Influenza B	0	0	0	0.0%
Influenza A swl (H1N1)	0	0	0	0.0%
Negative samples	—	—	4	5.3%

Bacterium	Single colonization	Viral/Bacterial pathogens	Total	%
Streptococcus pneumoniae	4	48	52	67.5%
Staphylococcus aureus	0	18	18	23.4%
Mycoplasma pneumoniae	0	4	4	5.2%
Haemophylus influenzae B	0	3	3	3.9%
Chlamydia pneumoniae	0	0	0	0.0%

pneumoniae. Spn detected in NAs were at low cycle-threshold values (under 25) in 50% of cases and these could be considered as an increased risk for bacterial pneumonia.

Among 23 hospitalized children with hRV infection, 20 (87%) were in the 2—6 month age group, all of which presented severe bronchiolitis. Identification of hRV alone in 9/23, and co-infections with BoV, CoVs, and hMPV required around 1—4 days of hospitalization; however, co-infections with AdV, RSV, EV, and PIV required longer hospitalization (4—10 days). Only one virus was detected in 7 out of 17 (41.2%) cases that required 6—18 days of hospitalization (Table 2).

Infections detected with RSV either alone in 8/14 of cases, or in co-infections with other respiratory viruses such as AdV, hRV, CoV, were more severe considering time of hospitalization (5—15 days). The median time of hospitalization in cases with RSV infection was 4.4 days, a 2-fold increase compared to cases with hRV infection (median time 2.2 days). Scientists have questioned whether hRV co-infections lead to more severe clinical syndromes, leading to longer hospitalizations.[10]

A better understanding of the full spectrum of bacterial colonization and of the respiratory viruses causing ARIs among hospitalized children in resource-limited tropical countries is essential to improve preventive and therapeutic strategies. It was suggested recently that co-infecting pathogens generally interact to worsen human health.[8] Platforms have not yet been adapted to the needs of clinics at district or local levels, nor have they even been in hospitals of major cities. To date, most surveillance for respiratory

Table 2 Distribution of cases by hospitalization days, intensive care necessity, and infectious agents.

Virus	Total	Days (hospitalization)	Intensive care	Co-morbidity
hRV alone	9	1—4	No	
RSV alone	8	2—12	No	
RSV/AdV	2	5—6	No	
RSV/hRV	1	14	No	
RSV/CoV	1	15	Yes	
RSV/PIV	1	2	No	
RSV/hRV/AdV	1	5	Yes	
hRV/EV	1	4	No	
hRV/EV/AdV/PIV	1	10	No	
hRV/AdV/PIV3/PIV4/CoV	1	4	No	
hRV/CoV	4	1—4	No	(1 case/10 days with cardiopathy)
hRV/CoV/BoV	2	2	No	
hRV/BoV	2	1/12	No/Yes	
hRV/BoV/hMPV	1	1	No	
hMPV alone	2	5	No	
hMPV/EV	1	18	No	Bronchopulmonary dysplasia

viruses in control programs are using an indirect immunofluorescence assay, detecting seven common respiratory viruses RSV, FluA and FluB, PIV1-3 and AdV. We describe for the first time in Paraguay the circulation of non-common respiratory viruses either alone or in co-infections with other respiratory viruses such as CoVs, BoV, hMPV and EV. The quantitative detection of multiple agents in nasopharyngeal specimens is an ideal approach to better understand the carriage and its relationship with the pathogen causing disease. Interpreting co-pathogen data is problematic due to the difficulty in differentiating acute disease from long-term shedding, and the extent that bronchiolitis may be caused or exacerbated by other viruses must be established.

Ethical approval

This study was approved by the Ethics Committee of the Instituto de Investigaciones en Ciencias de la Salud, the Universidad Nacional de Asunción (IICS-UNA), and the Hospital Pediátrico Niños de Acosta Ñu.

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Competing interests

The authors have no competing interests with regard to the contents of this manuscript.

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