

Characterization of respiratory infection viruses in hospitalized children from Naples province in Southern Italy

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Abstract. Most acute respiratory infections (ARIs) in children are due to viral etiology, and represent an important cause of mortality and morbidity in children <5 years old in developing countries. The pathogens that cause ARIs vary geographically and by season, and viruses serve a major role. In the present study, the distribution of the seven respiratory viruses that are more prevalent in Southern European countries were retrospectively analyzed in a Southern Italy Hospital, that centralizes pediatric diseases from the Naples province. Viruses were categorized by a FilmArray Respiratory Panel, and demonstrated no substantial differences in sex, age and seasonal viruses distribution. However, all the investigated viruses had a higher detection rate in the surrounding municipalities than in the metropolitan area of Naples. In recent years, the association between air pollution and respiratory infections has become an increasing public health concern. The data in this study support this association in the surrounding areas of Naples extensively contaminated by environmental toxic agents. In these areas, characterization of the epidemiology of ARIs is required to implement a prevention and control program.

Introduction

Acute respiratory infections (ARIs) represent a health issue of great importance, leading cause of mortality in children worldwide, particularly in developing countries (1). These represent

about 50% of all diseases in children aged <5 years (2). The major viral agents of ARI include influenza A, B, and C viruses (FLU), respiratory syncytial virus (RSV), parainfluenza virus (PIV), adenovirus (ADV), human metapneumovirus (hMPV), human coronavirus (HCoV), and Rhinovirus (HRV).

The etiology of respiratory diseases is multifactorial and includes, among others, interactions between genetic predisposition and environmental factors (3). Numerous studies have confirmed that short- and long-term exposures to ambient air pollutants can be associated with a wide range of pathologies, in particular respiratory diseases and cancer. Although air pollution has not been shown as the sole cause of respiratory infections, it has been reported that several air pollutants were correlated with increased morbidity of respiratory infections (4). In urban areas, irrespective of seasonal frequencies, this correlation increases due to the high incidence of polluting factors. Several epidemiological studies have documented a positive association between exposure to particulate air pollution and respiratory symptoms especially among children (5-7).

It is profusely reported that during the past three decades, large areas of Naples county in the Southern Italy Campania region have been extensively contaminated by environmental toxic agents, in particular for the presence of many landfills of industrial wastes. This region was already defined as one of the geographical areas most at risk of neoplastic and respiratory diseases for environmental factors in the report of WHO (World Health Organization) in 1997 (8).

Although, the epidemiological studies for the association between air pollution and the incidence of respiratory infection in this geographical area are relatively few, virus types identification and their molecular characterization is fundamental not only for surveillance, for diagnostic and therapeutic purposes, but also for highlight the relationships between respiratory diseases in children and concentrations of environmental pollutions (9).

In the present study we characterized the viral spectrum and pattern of ARIs in children from children's hospital 'Santobono Pausilipon' which centralizes pediatric diseases of the entire Naples province. The aim of this study was to determine the association between respiratory viruses types, patients features (sex, age, season of disease occurrence) and, in particular, geographical origin.

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Materials and methods

Patients selection. We retrospectively reviewed the electronic medical records of 356 patients between 11 days and 14 years, with suspected respiratory infection, evaluated at the Santobono Hospital of Naples, between 1 January 2016 and 31 January 2017, with FilmArray® Respiratory Panel (FARP) testing on NasoPharyngeal Swab (NPS).

From patient electronic medical records the following information was obtained: Demographics (age and sex), month of hospitalization, and geographical origin.

We have divided the patients into three age groups (Early childhood, 0-2 years; preschool age, 2-5 years; third childhood: 5-11 years), in the season of hospitalization (winter: December, January, February; spring: March, April, May; summer: June, July, August; autumn: September, October, November) and geographical origin (metropolitan area of Naples and extra-urban areas).

FilmArray testing. NPSs were collected according to a standard procedure, kept in viral transport medium, and stored at -20°C prior to analysis.

FARP (FilmArray® Respiratory Panel BioFire Diagnostics LLC 390; Wakara Way Salt Lake City, UT, USA) is a test based on multiplex PCR. The FilmArray RP cartridge is designed for the simultaneous detection and identification of following viruses and bacteria of the upper respiratory tract: Influenza A virus (H1N1, H1N1 2009, and H3N2), influenza B virus, RSV, PIVs 1-4, ADV, HRV/enterovirus (the assay does not distinguish between these two pathogens), HMPV, HCoV (229E, HKU1, OC43, and NL63), *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Bordetella pertussis*. The FilmArray instrument and pouch system have been described in detail elsewhere (10). The research use only version of the FilmArray RP system reported a cycle threshold for each positive PCR assay (11).

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Viral prevalence were compared using the Chi-square test for categorical variables, and the cartogram was drawn using Excel software (Microsoft Corporation, Redmond, WA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Demographic characteristics. A total of 356 nasopharyngeal swabs were collected and analyzed. In detail, 163 patients were female (45.78%) and 193 were male (54.21%). Most of the patients (319) were <5 years old while 36 patients were >5 years old. Patients are heterogeneously distributed in different seasons, while regarding geographical origin, 123 (37.9%) patients are of Naples metropolitan area, 201 (62.03%) originated from neighboring municipalities, and for 32 patients this information is lacking.

In our study we considered only viral infections, but we detected also other etiologic agents (*Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*), highlighted the presence of these infections in 24 patients (6.7%).

Respiratory infection viruses distribution

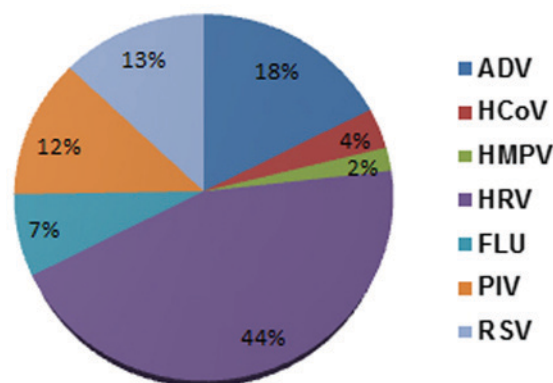


Figure 1. Respiratory infection viruses distribution in hospitalized children.

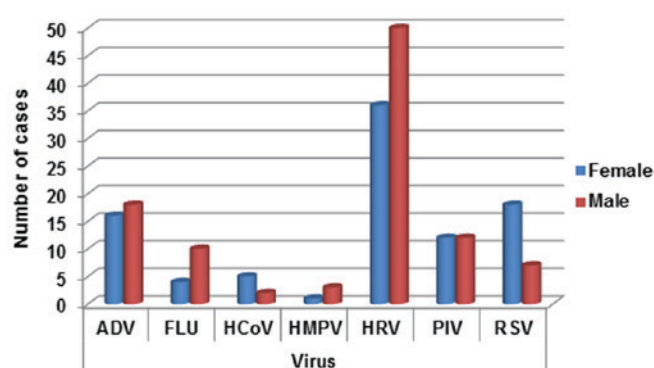


Figure 2. Respiratory infection viruses distribution in male and female patients. ADV, adenovirus; FLU, influenza A, B, and C viruses; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Respiratory infection viruses distribution. The total rate of detection of all seven viruses was 78% (278/356) of patients. In detail, HRV viruses were detected in 44% of patients, followed by ADV viruses (18%), RSV viruses (13%), and PIV (12%). A lower incidence has been instead highlighted for FLU (7%), HCoV (4%) and HMPV (2%) (Fig. 1).

In a significant proportion of individuals co-infections were also highlighted. In detail, double co-infections were detected in 69/256 (26.9%) patients, triple in 17/256 (6.6%) and quadruple infections only in 1 patient (Table I). The more frequent association was detected between HRV and PIV viruses 19/87 (21%), followed by HRV and ADV viruses 15/87 (17.24%), and HRV and RSV viruses 9/87 (10.3%) (Table I).

Sex and age distribution. Viruses appear heterogeneously distributed between sex, with the exception of PIV. In detail, ADV was slightly higher in males (18/34, 52.9%), while FLU (10/14, 71.4%), HMPV (3/4, 75%) and HRV (50/86, 58.13%) were strongly higher in males children (see detection rate Fig. 2). On the contrary HCoV (5/7, 71.4%) and RSV (18/25, 72%) are better represented in female patients (see Fig. 2).

Regarding age distribution a decline in the incidence of viral infections with age was observed for respiratory viruses, except for FLU (Fig. 3). In fact the detection rate for FLU viruses was lowest in 2-5 years patients. The detection rate for

Table I. Co-infections with seven respiratory viruses.

Co-infections	N
HRV + PIV	19
HRV + ADV	15
HRV + RSV	9
HRV + ADV + PIV	4
ADV + PIV	4
HRV + HMPV	4
HRV-ADV + HCoV	3
ADV + FLU	3
RSV + HCoV	3
ADV + HCoV	2
FLU + RSV	2
HRV + HCoV	2
HCoV + PIV	2
RSV + ADV + HMPV	2
HRV + RSV + ADV	2
RSV + HMPV	2
HRV + RSV + PIV	2
FLU + PIV	1
HRV + FLU	1
HRV + HCoV + PIV	1
RSV + ADV + HRV + PIV	1
RSV + ADV + HCoV	1
HRV + RSV + HCoV	1
HRV + RSV + HMPV	1

FLU, influenza A, B, and C viruses; RSV, respiratory syncytial virus; PIV, parainfluenza virus; ADV, adenovirus; hMPV, human metapneumovirus; HCoV, human coronavirus; HRV, rhinovirus.

ADV gradually decrease from 0-2 years patients to >5 years patients. The same trend was highlighted for HCoV, PIV, HRV and RSV (with a consistently increasing of detection rate in 0-2 year-old patients). HMPV was detected prevalently in 0-2 year-old children (Fig. 3).

Seasonal distribution. The total detection rate for all respiratory viruses in spring, summer, autumn and winter was calculated. The rates of detection was more heterogeneous during the different seasons.

In detail, ADV infection was prevalent in summer (20/34, 58.8%) and spring (10/34, 29.4%) seasons, FLU was prevalent in spring (7/14, 50%) and winter (4/14, 28.5%), HMPV in winter (2/4, 50%), HCoV in autumn/winter (3/7, 42.8%), HRV in autumn (34/86, 39.5%) and summer (30/86, 34.8%), PIV in summer (13/24, 34.8%) and RSV in winter (24/25, 96%) (Fig. 4).

Geographical distribution. The pediatric patients were divided into two main groups according to origin from the metropolitan of Naples and neighboring municipalities. The detection rates for the two areas appear different with a prevalent distribution in extra-urban areas. This appears more evident especially for ADV, HRV and RSV viral infections (Fig. 5).

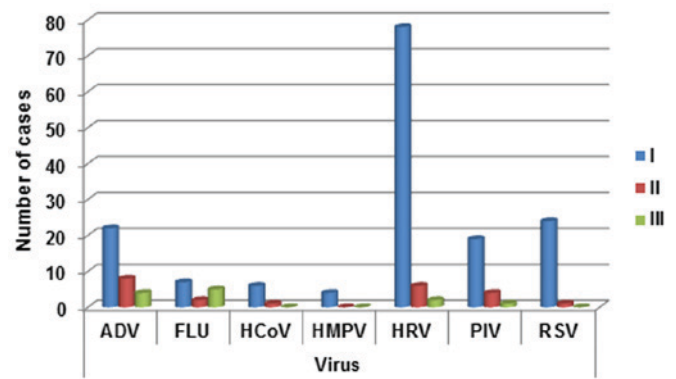


Figure 3. Respiratory infection viruses distribution in the three age groups (0-2 years, 2-5 years, >5). ADV, adenovirus; FLU, influenza A, B, and C viruses; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

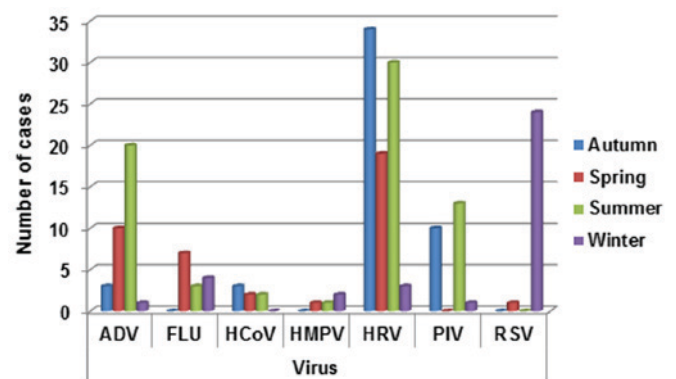


Figure 4. Respiratory infection viruses distribution by season (winter: December, January, February; spring: March, April, May; summer: June, July, August; autumn: September, October, November). ADV, adenovirus; FLU, influenza A, B, and C viruses; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

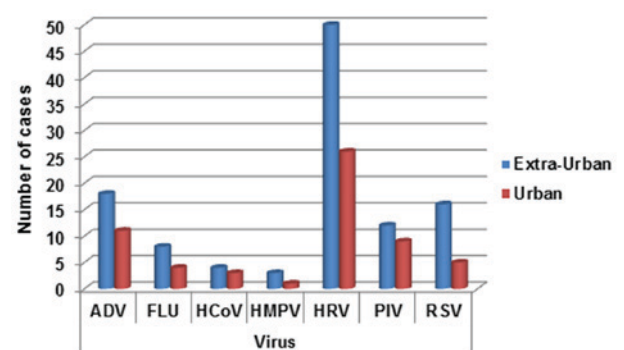


Figure 5. Respiratory infection viruses distribution for territorial origin (urban, Naples metropolitan area; extra-urban, neighboring municipalities). ADV, adenovirus; FLU, influenza A, B, and C viruses; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Discussion

WHO reported that ARI can represent among the leading causes of mortality in children under 5 years of age.

Many studies in the literature have described in detail the viruses mainly associated with ARI and also their distribution, but showing much conflicting data on populations from different countries (1). These variations may be due to socio-economic factors, geographical and climatic differences and efficiency of local health care systems.

In recent years, advances in PCR techniques have aided in the rapid and accurate detection of common respiratory pathogens from patient specimens. Multiplex PCR can identify and differentiate a large panel of viral and bacterial targets simultaneously and are more rapid and more sensitive methods than cultures or antigen detection (12,13).

In the present report we analyzed the distribution of seven respiratory viruses in a case series of 356 hospitalized patients in metropolitan area of Naples and in the neighboring municipalities. The incidence of all analyzed virus is prevalent in early childhood. The distribution of viruses, considered individually, appears to be very heterogeneous, with the largest percentage of HRV in line with other studies on different populations (14,15). HRV (including Rhinovirus and Enterovirus) are RNA viruses related to *Picornavirus* family (16). HRV is associated with the common cold, but can also be implicated in exacerbating asthma attacks and severe complications (15). The Enteroviruses are categorized into four species, which include a total of 89 serotypes associated with different clinical manifestations (16,17).

In our case series HRV appears more expressed in the male population and its seasonality is mainly associated with summer and autumn, in line with the data present in the literature (16,18).

RSV and ADV are, after HRV, the most common virus in our case series, with RSV prevalent among the female children and ADV among the male population.

RSV is a member of the *Paramyxoviridae* RNA viruses family related to human metapneumovirus and PIVs. RSV is the most common cause of severe respiratory illness in infants with acute bronchiolitis as a leading cause of hospitalization (19).

In our case series the winter seasonality of RSV viruses corresponds to the other reported data (19).

ADV is a DNA virus uncoated consists of seven species (A-G) and classified by hemagglutination with about 55 serotypes. The species of ADV B, C and E cause acute respiratory disease with the main risk factor the long stay indoors (18) while the ADV (species A, D, F and G) can cause cystitis, gastroenteritis and conjunctivitis (20).

In our study the seasonal distribution of ADV is prevalent in summer in line with the literature (21).

While there are no substantial differences in PIV distribution, FLU seems prevalent in the male population and their seasonality is consistent with data reported in the literature (22). Finally, HCoV and hMPV viruses are less frequent in our population, with the first more present in male pediatric population and hMPV in female children.

Coronavirus HCoV are characterized 4 serological variants (229E, HKU1, NL63 and OC43), and are most commonly associated with infections of the upper respiratory tract (23).

hMPV belong to the *Paramyxoviridae* family and the infection in newborns and young children is commonly associated with bronchiolitis (24).

It is also widely documented the occurrence of co-infections in some cases. In our case series the more frequent co-infection are between HRV and PIV viruses and HRV and ADV viruses. These data, in some cases, contrast with other reports (25). However, this might be closely related to the geographical location, climate and different social and socio-cultural conditions.

Co-infections were more common in pediatric patients than in adults as documented by other studies (26-28).

However, multiple viral infections can be linked to hospital stay, abuse of antibiotic and social conditions, but there are not proves that co-infections can worsen the disease course. Our hospital centralizes the majority of patients coming from the metropolitan area of Naples but also from neighboring municipalities. Our data are interesting for the geographic distribution of patients. In fact all the investigated viruses have a detection rate higher in surrounding municipalities than in the metropolitan area of Naples. This is in contrast with most of the data present in the literature, where the prevalence is just in urban areas due to the high presence of pollutants (29-31).

However, during the past three decades, surrounding areas of Naples have been extensively contaminated by environmental toxic agents, in particular for the presence of many landfills of industrial wastes. The most contaminated areas were defined as 'Land of Fire' (32).

The relationship between air pollution and respiratory infections has become an increased public health concern in recent years (33-35). In fact, the etiology of respiratory diseases is multifactorial and includes, among others, interactions between genetic predisposition and environmental factors as climate change, chemical air pollution and airborne pollens. The short-term respiratory effects of air pollution include decreases in pulmonary function (36), increases in inflammatory biomarkers and respiratory symptoms (37), infections (38), and respiratory mortality (39).

The environmental risk factors may have an impact on children's respiratory health, above all in urban areas, especially because children inhale more pollutants per kilogram of body weight than adults (40).

In conclusion, the risk factors between populations can be extremely different, suggesting the need to adequately characterize epidemiology of ARIs to implement prevention and control program.

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