

Epidemiology of 11 Respiratory RNA Viruses in a Cohort of Hospitalized Children in Riyadh, Saudi Arabia

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Respiratory tract infections are a principal cause of illness and mortality in children worldwide and mostly caused by viruses. In this study, the epidemiology of 11 respiratory RNA viruses was investigated in a cohort of hospitalized children at a tertiary referral center in Riyadh from February 2008 to March 2009 using conventional and real-time monoplex RT-PCR assays. Among 174 nasopharyngeal aspirates, respiratory syncytial virus (RSV) was detected in 39 samples (22.41%), influenza A virus in 34 (19.54%), metapneumovirus (MPV) in 19 (10.92%), coronaviruses in 14 (8.05%), and parainfluenza viruses (PIVs) in 11 (6.32%). RSV, PIVs and coronaviruses were most prevalent in infants less than 6 months old, whereas MPV and influenza A virus were more prominent in children aged 7–24 and 25–60 months, respectively. The majority of the viruses were identified during winter with two peaks observed in March 2008 and January 2009. The presented data warrants further investigation to understand the epidemiology of respiratory viruses in Saudi Arabia on spatial and temporal basis. *J. Med. Virol.* **88:1086–1091, 2016.** © 2015 Wiley Periodicals, Inc.

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The socio-economic burden of RTIs also includes medication and vaccination costs, loss of work and school hours and effect on individuals' productivity.

The etiology of RTIs is complex and the identification of the primary causative organism is quite difficult [Kouni et al., 2013] because of the following reasons: (i) the involvement of large number of pathogens in the disease syndrome, (ii) the lack of specific clinical symptoms linked to particular causative agents, (iii) the mixed infection, with up to six respiratory pathogens, is not an uncommon observation [Martin et al., 2012]. In almost two-thirds of RTIs in children, viruses are convicted as the principal cause. The most common respiratory viruses involved in are: respiratory syncytial virus (RSV), influenza A and B viruses, and parainfluenza viruses (PIVs). Recently, the importance of other viruses like metapneumovirus (MPV) and coronavirus (CoV) becomes evident [Mackie, 2003]. The degree of involvement of each respiratory virus in the annual disease outbreaks varies significantly depending on the geographical location, climatic conditions, season, demographic patterns and health care system.

The population of Saudi Arabia is particularly under great risk of exposure to a wide diversity of wild-type and mutant strains of different respiratory viruses. In addition to the impact of mass gathering during Hajj season, Saudi Arabia represents one of the major countries that accept foreign workforce

Respiratory tract infections (RTIs) are the major cause of illness, hospitalization, and mortality in children less than 5 years old worldwide [Johansson et al., 2011]. Recent records have indicated that RTIs kill 1.5–2.1 million children annually with 90% of these deaths are caused by pneumonia and bronchiolitis [Black et al., 2010]. The World Health Organization categorizes RTIs as the second primary cause of death in this age-group globally [Bryce et al., 2005].

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from a diverse of countries worldwide. The regular travelling of workforce to Saudi Arabia and their return back to their home countries provides the mean of renewing the pool of circulating respiratory viruses each year. Despite the significant danger of RTIs to Saudi population, there is a lack of research in this field. Few reports have described the prevalence of certain respiratory viruses and the impact of a limited number of risk factors among hospitalized children in Riyadh, Abha, Qassim, and Najran during scattered epidemic seasons [Al-Shehri et al., 2005; Al-Majhdi et al., 2009; Bukhari and Elhazmi, 2013; Al-Ayed et al., 2014].

This study is a cross-sectional analysis for the prevalence of respiratory viruses responsible for acute RTIs in a cohort of hospitalized children at King Khalid University Hospital (KKUH) over a 14 month period from February 2008 till March 2009. KKUH is an 800-bed facility that locates in Riyadh and provides tertiary care services to all Saudi citizens on referral bases. The included subjects were neonates, infants and young children less than 5 years of both sexes. All patients presented at least two signs of acute respiratory tract infection including: cough, fever, rhinorrhea, dyspnea, sore throat, myalgia, wheezing, and respiratory distress [Bierbaum et al., 2014]. The exclusion criteria for this study were patients with immunosuppression, malignancy and autoimmune disease, and those receiving antiviral chemotherapy. Pertinent demographic and clinical information such as age, gender, ethnicity, onset and types of symptoms, underlying disorders, and vaccination history were obtained from the hospital records (Table I). The study protocols conformed to the 1975 Declaration of Helsinki and were approved by the Ethical Committee of King Saud University. Nasopharyngeal aspirate (NPA) samples

were collected from hospitalized patients by a qualified nursing staff after getting informed consents from their legal guardians [Heikkinen et al., 2002]. In few cases, sputum samples were collected and prepared by standard methods [Zar et al., 2000].

Viral RNA was isolated from 140 μ l volumes of the clinical specimens using QIAamp Viral RNA extraction kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA was dissolved in 60 μ l elution buffer and stored in separate aliquots for use in virus detection. Monoplex gel-based RT-PCR assays were performed for the detection and typing of RSV, influenza and parainfluenza viruses using one-step RT-PCR kit (Qiagen) and the primer sets listed in Supplementary Table SI. The reaction mixture was made up of 10 μ l of RNA, 1 \times Qiagen one-step RT-PCR buffer, 0.4 mM of each dNTP, 0.6 μ M of sense and antisense primers, and 1 μ l of Qiagen one-step RT-PCR enzyme mix. Amplification was accomplished in GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) using the following reaction setup: cDNA synthesis at 50°C for 30 min; initial PCR activation at 95°C for 15 min; 35–40 cycles of denaturation at 94°C for 30 sec, annealing at 50°C (PIVs and influenza viruses) or 52°C (RSV) for 30 sec, and polymerization at 72°C for 1–2 min; and final extension at 72°C for 10 min. RT-PCR products were separated in 1.5% agarose gel and were visualized using IMAGO imaging system (B&L systems, Netherlands). Genesig standard one-step real-time RT-PCR kits (PrimerDesign, Southampton, UK) were used to detect HMPV and human coronaviruses of group 1b (NL63 and E229) and group 2a (OC43 and HKU1). The assays were carried out separately according to the manufacturer's protocol on ABI Prism 7500 Sequence Detection System (Applied Biosystems). Samples with C_T values higher

TABLE I. Demographic Characteristics of Patients With Positive Screening Results as Classified by Virus Type

| Variable | RSV (%) | Influenza A (%) | PIVs (%) | MPV (%) | CoVs (%) | Mixed (%) | Total (%) |
|-------------------------------|------------|-----------------|-----------|------------|------------|-----------|------------|
| Gender (%) | | | | | | | |
| Male | 26 (66.7) | 15 (44.1) | 7 (63.6) | 14 (73.7) | 8 (57.1) | 7 (58.3) | 63 (60.0) |
| Female | 13 (33.3) | 19 (55.9) | 4 (36.4) | 5 (26.3) | 6 (42.9) | 5 (41.7) | 42 (40.0) |
| Age | | | | | | | |
| 0–6 months | 19 (48.7) | 11 (32.4) | 5 (45.5) | 2 (10.5) | 7 (50.0) | 6 (50.0) | 38 (36.2) |
| 7–24 months | 15 (38.5) | 11 (32.4) | 4 (36.4) | 13 (68.4) | 6 (42.9) | 2 (16.7) | 47 (44.8) |
| 25–60 months | 5 (12.8) | 12 (35.2) | 2 (18.2) | 4 (21.1) | 1 (7.1) | 4 (33.3) | 20 (19.0) |
| Ethnicity | | | | | | | |
| Saudi | 39 (100.0) | 33 (97.1) | 10 (90.9) | 19 (100.0) | 14 (100.0) | 11 (91.7) | 104 (99.0) |
| Non-Saudi | 0 (0.0) | 1 (2.9) | 1 (9.1) | 0 (0.0) | 0 (0.0) | 1 (8.3) | 2 (1.9) |
| Disease form | | | | | | | |
| Upper RTI | 0 (0.0) | 10 (29.4) | 1 (9.1) | 1 (5.3) | 9 (64.3) | 1 (8.3) | 20 (19.0) |
| Lower RTI | 39 (100.0) | 24 (70.6) | 10 (90.9) | 18 (94.7) | 5 (35.7) | 11 (91.7) | 85 (81.0) |
| Underlying disease conditions | | | | | | | |
| Bronchial asthma | 20 (51.3) | 2 (5.9) | 3 (27.3) | 2 (10.5) | 0 (0.0) | 1 (8.3) | 26 (24.8) |
| Cardiopulmonary disease | 5 (12.8) | 1 (2.9) | 2 (18.2) | 3 (15.8) | 0 (0.0) | 0 (0.0) | 11 (10.5) |
| Vaccination history (Flu) | | | | | | | |
| Vaccinated | 11 (28.2) | 1 (2.9) | 1 (9.1) | 6 (31.6) | 2 (14.3) | 3 (25) | 18 (17.1) |
| Non-vaccinated | 28 (71.8) | 33 (97.1) | 10 (90.9) | 13 (68.4) | 12 (85.7) | 9 (75) | 87 (82.9) |

RTI, respiratory tract illness/infection.

than or equal to 40 were considered negative, while those higher than 35 and less than 40 were re-tested for confirmation.

Data were entered into spreadsheet databases using Microsoft[®] Excel and cleaned from abnormal figures. Values were expressed as percentages and frequencies for discrete/categorical variables. For continuous variables, descriptive statistics like mean, median and standard deviation (SD) were utilized. Chi-squared test was used for identification of statistical significant values (P -values <0.05).

A total of 174 NPAs were collected from patients that met the inclusion criteria of the study and were analyzed for the presence of 11 respiratory viruses including RSV, influenza A and B viruses, parainfluenza viruses 1, 2 and 3, MPV, human coronavirus (HCoV) NL63, E229, OC43, and HKU1. At least one respiratory virus was identified in 105 (60.34%) of the samples, while 69 samples (39.66%) were negative. The most frequently detected virus was RSV ($n=39$; 22.41%), followed by influenza A virus ($n=34$; 19.54%), MPV ($n=19$; 10.92%), human coronavirus (HCoV) OC43 and HK1 ($n=13$; 7.48%); PIV-3 ($n=10$; 5.75%), PIV-2 ($n=1$; 0.57%), and HCoV-E229 and HCoV-NL63 ($n=1$; 0.57%). Influenza B virus and PIV-1 were never detected in all samples. Among RSV positive samples, 23 (59%) were type A and 16 (41%) were type B. The majority of positive samples ($n=96$; 88.57%) contained only one respiratory virus, whereas 12 (11.43%) samples were positive for two viruses. Influenza A virus was the most common pathogen in mixed infections ($n=9$) followed by RSV ($n=8$), while CoVs were not identified in any of these cases.

Patients' data available at the hospital records were analyzed for potential association of the different risk factors and the causative organism. Significantly more positive samples were identified in males ($n=63$; 60.0%) than in females ($n=42$; 40%); $P=0.04$. In particular, RSV and MPV positive samples showed this significant difference ($P=0.037$ and 0.039 respectively). However, the variation for the rest of viruses was insignificant albeit the rate of detection in males was always higher than in females except for influenza A virus (P value ranged from 0.49 to 0.63) (Table I).

All children enrolled for this study aged 10 days to 5 years and were distributed into three age groups: 0–6 months ($n=59$; 33.9%), 7–24 months ($n=79$; 45.4%), and 24–60 months ($n=36$; 20.7%). The frequency of positive samples in each age group is well correlated with the overall percentage with a prevalence of 35.2%, 43.8% and 20.9% in order. RSV was the most common virus identified in patients younger than 6 months accounting for 43.2% of positive cases ($P=0.018$). In the other age groups, the incidence of RSV infection decreased steadily and represents 30.6% and 20.8% of the cases respectively. PIVs and CoVs have the same age distribution of RSV with less significance ($P=0.529$ and

0.109, respectively), whereas MPV appears to dominate in children aging from 6 to 24 months ($P=0.004$). The prevalence of influenza A virus in the first two age groups was moderate, while it constitutes 50% in patients older than 2 years (Tables I and II).

Since all of the patients were hospitalized, the majority were suffering from symptoms of lower RTI; mostly bronchitis and pneumonia (81%). About 24.8% of the cases were accompanied with bronchial asthma and 10.5% are complicated with cardiopulmonary disease. No specific relationship between the disease severity and the virus cause was observed except for RSV in which a higher rate of complications was correlated. The effect of ethnicity was not considered in this analysis since all but two patients were Saudi and they all represent the mixed population of Riyadh. Similarly, the vaccination history did not provide sufficient information for investigation due to the limited number of vaccinated patients and the inability of most patients to give an accurate data (Table I).

The temporal prevalence of the respiratory viruses in the study group was evaluated based on the admission dates for the affected children in the hospital wards. As expected, the admission rate was variable during the surveillance period with a greater increase in the winter seasons (particularly between January and March); $P=0.0002$. The rate of detection of respiratory viruses was insignificant relevant to the periods of hospitalization ($P=0.84$) with two obvious peaks at March 2008 and January 2009. March and December 2008 were the months that exhibit higher prevalence of positive samples with an incidence of 87.5 and 70%, respectively. Similar peak patterns were shown for RSV and MPV with a lower infection rate of the latter. Influenza A demonstrated a distinct peak at March 2008 and a small peak at February 2009. Coronaviruses prevailed at the last

TABLE II. Distribution of the Detected Viral Agents According to the Age Group

| Virus | Age range | | |
|-------------------------------|-----------------------|---------------------------|-------------------------|
| | 0–6 months No. (%) | 7–24 months No. (%) | 25–60 months No. (%) |
| Respiratory syncytial viruses | 19 (43.2) | 15 (30.6) | 5 (20.8) |
| RSV-A | 11 (25) | 9 (18.4) | 3 (12.5) |
| RSV-B | 8 (18.2) | 6 (12.2) | 2 (8.3) |
| Influenza A viruses | 11 (25) | 11 (22.4) | 12 (50) |
| Parainfluenza viruses | 5 (11.4) | 4 (8.3) | 2 (8.3) |
| PIV-2 | 0 (0) | 1 (2.1) | 0 (0) |
| PIV-3 | 4 (9.1) | 3 (6.2) | 2 (8.3) |
| Metapneumovirus | 2 (4.5) | 13 (26.5) | 4 (16.7) |
| Coronaviruses | 7 (15.9) | 6 (12.2) | 1 (4.2) |
| CoV-1b | 1 (2.3) | 0 (0) | 0 (0) |
| CoV-2a | 6 (13.6) | 6 (12.2) | 1 (4.2) |
| Mixed Infection | 6 (13.6) | 2 (4.1) | 4 (16.7) |
| Total | 44 | 49 | 24 |

months of the surveillance period with a distinct peak at February and March 2009. PIVs did not show any temporal peaks and were evenly distributed throughout the study course (Fig. 1).

Recognition of the spectrum and diversity of respiratory viruses in Saudi population is critical not only for determining appropriate disease control measures in Saudi Arabia, but it also provides an early alarming system for emerging viruses worldwide. In the current study, the respiratory viruses were detected in 60.34% of a cohort of hospitalized children at KKUH in Riyadh. According to the best of our knowledge, this rate of detection is the highest of all studies conducted in Saudi Arabia [Al-Shehri et al., 2005; Bukhari and Elhazmi, 2013; Al-Ayed et al., 2014]. The relative increase in the detection level is possibly a result of using highly sensitive molecular techniques, the inclusion of more viruses in the diagnostic array, and/or the retrieval of samples only from hospitalized symptomatic patients.

RSV was the most commonly detected virus with an incidence of 22.41% (37.14% of positive samples). It is well-documented that RSV contributes in the majority of acute RTIs that requires hospitalization in children of pre-school age [Collins and Crowe,

2007]. The worldwide prevalence of RSV infection in this age group varies widely depending on geographical and chronological factors but mostly ranges from 14.1% [Kwofie et al., 2012] to 50% [Meqdam et al., 1997]. In Saudi Arabia, the diversity in occurrence is much profound with limits that extend from 24.4% [Al-Ayed et al., 2014] to 95.5% [Bukhari and Elhazmi, 2013] in positive samples, which may reflect bias in sample collection. Not only the prevalence of RSV in this study matches the national and international figures, but it also reflects the dominance of type A viruses in most communities and seasonal outbreaks [Zhang et al., 2007]. For the prevalence of other respiratory viruses in the study group, it seems that all of them lie in the upper worldwide expected range. It is notable that no previous records in Saudi Arabia have shown a prevalence of MPV and CoVs in positive samples exceeding 9.6% and 2.96% respectively [Al-Ayed et al., 2014] [compare with 18.1% and 13.3% in this study].

Analyzing the effect of risk factors on the prevalence of respiratory viruses detected in the current study population indicated several remarkable features linked to gender, age and season (Table I). Male gender was more susceptible to RTI of viral

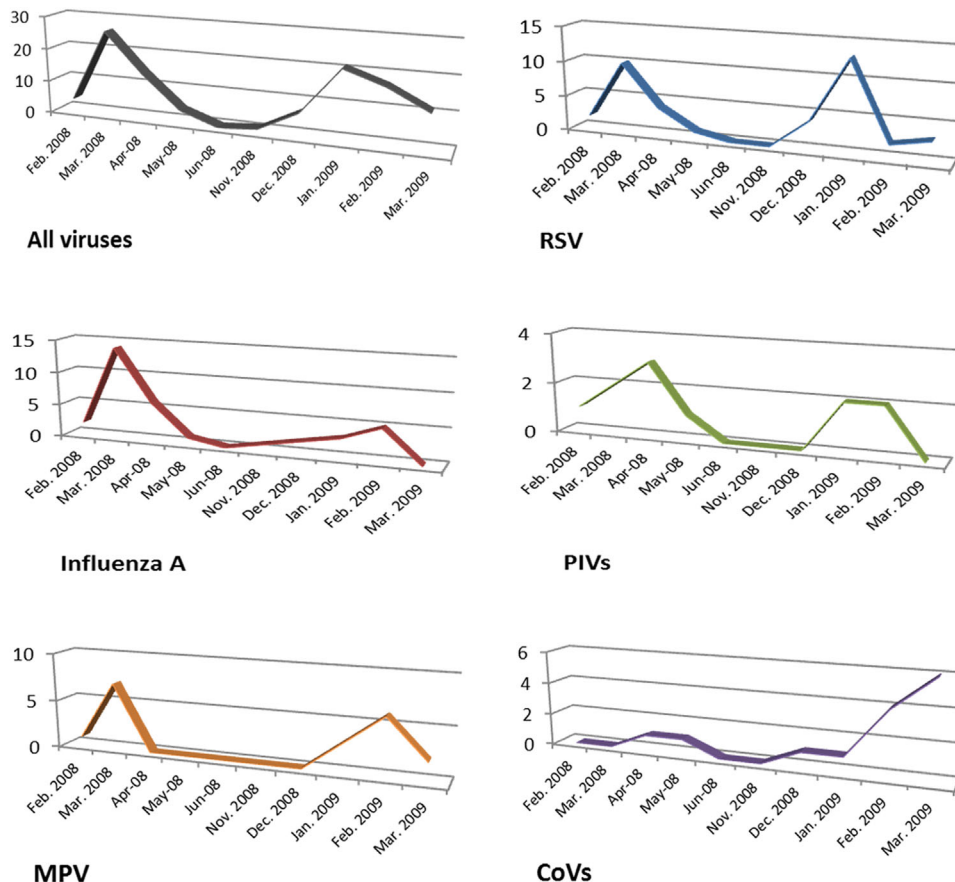


Fig. 1. Monthly variation of respiratory viruses from February 2008 to March 2009. X-axis define the month and Y-axis shows the number of cases.

origin as compared to female gender. This higher susceptibility of males was also demonstrated for individual viruses except for influenza A virus. It is evident from the literature that males are always at greater risk of lower RTI with more severe course and higher mortality rate in all age groups [Beka et al., 2013]. The anatomical difference in the respiratory airways of boys that are narrower and shorter than their counterpart of girls may partially justify the higher susceptibility of males [Meissner, 2003]. A significant association was demonstrated between RSV infection and younger age group (0–6 months) with a prevalence of 43.2% and between influenza A virus infection and older age group (24–60 months) with a prevalence of 50% (Table II). This pattern is well-established through the results of many studies in Saudi Arabia [Bakir et al., 1998] and internationally [Karadag-Oncel et al., 2014].

There is a distinct seasonality of the different respiratory viruses with clear peak(s) in certain seasons and months. In temperate and desert climates, infection with respiratory viruses appears in colder months (November–March). Outbreaks of RSV and MPV start at October/November with a clear peak at January/February, while influenza virus activity begins earlier at September/October and peaks at November/December. Except for PIV-2, PIVs are mostly seen at spring and summer seasons with no obvious peak activity [Bakir et al., 1998]. Relative conclusions could be obtained from this study, where RSV and MPV peaks appeared at March and January of two successive years, influenza peaks appeared at March and February, while PIVs had no distinct peak activity (Fig. 1). Start of sample collection at February 2008 and retrieval of a limited number of samples between May and December 2008 essentially affected the interpretation of results on timely manner with more or less shift in the peak activity of certain viruses.

Although the current report may add valuable information that improves the knowledge on the respiratory viruses circulating among Saudi population, some limitations should be addressed: (i) the number of samples is somewhat limited and may not offer a complete reliability to compare with certain risk factors; (ii) the samples are all driven from a motivated study population (hospitalized children), (iii) the overall frequency of respiratory viruses in the study population could not be estimated since certain viruses such as rhinovirus, bocavirus and adenovirus were not tested, (iv) the restrictive available clinical and demographic data, due to administrative, social and cultural reasons, has affected complete consideration of the factors that may predispose the disease syndrome in the Saudi community. In conclusion, this study provides preliminary data on the prevalence and epidemiology of respiratory viruses that affect young children in Riyadh province (Saudi Arabia). Further analysis of similar cohorts/populations on temporal and spatial levels will provide

better understanding of disease predisposition, circulation, and progression in Saudi Arabia.

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