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Original Article

Respiratory virus infection among the children below 2 years in Nepal 2019- A retrospective cross-sectional study

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ABSTRACT

Background: Acute respiratory infections of viral and bacterial origin pose serious problems owing to their great prevalence, with associated high morbidity and mortality. In South Asia, acute respiratory acute respiratory infections cause about 15% of all pediatric deaths. Although infection rates are reported annually, there is limited information available regarding circulating respiratory pathogens. The aim was to determine the etiological agents of these respiratory diseases using multiplex molecular tests aiming to enhance knowledge on the circulation of viruses that causes acute respiratory infections in Nepal.

Materials and Methods: This was a laboratory based retrospective cross-sectional study conducted from December 2020 to March 2021 on archived samples at National Influenza Center, Nepal, among children ≤2 years, who gave sample for influenza testing by the Dolphin system.

Results: Of the 80 samples, 46 were positive. The most common was Respiratory Syncytial Virus A/B:13, followed by Adenovirus:12, Influenza A:9, M. Pneumonia:4, ParainfluenzaVirus-3:4, Human Metapneumovirus Virus A/B, Human parechovirus, Rhinovirus, Influenza B:3, Para Influenza virus 4, Para Influenza Virus 2, Para Influenza virus 1 & Coronavirus 43:1. Also our result showed coinfectionin 11 samples of total tested samples.

Conclusions: With a limited sample size, this study shows evidence of different pathogens that cause acute respiratory infection thus suggesting the need for community surveillance to enhance public health efforts and scientific understanding of respiratory infection for appropriate prevention, treatment & management strategies through strengthening molecular diagnostic methods which provide a rapid, sensitive and specific approach to improve the etiological diagnosis of infection.

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INTRODUCTION

Acute respiratory infections (ARIs), continue to be one of the persistent public health problems that cause of morbidity and mortality worldwide.[1] The incidence of ARI is high among under-five children and leading cause of death in children, especially in developing countries.[2,3] These are responsible for hospitalization due to respiratory diseases in children population mostly in infants under 1-year.[4,5] Globally it is estimated that 3.9 million[6] children die annually due to respiratory tract infections mainly in the African and Southeast Asian countries.[2] According to the annual

Department of Health Service (DoHS) report-2076/2078, Nepal records on an average 30,13,891 under 5 children were estimated to have been prone to ARI with a case fatality rate of 0.13[7]. However, the data on ARI from children below 2 years are scarce.

The etiology of ARIs includes both bacteria and viruses. Mainly viral etiology is responsible for 80% of cases and is associated with symptomatic respiratory tract infection in developing countries, especially among children under 5 years[3,8], such as Respiratory Syncytial viruses (RSVA/B), Human parainfluenza viruses (HPIV), Influenza virus A & B, human metapneumovirus (HMPVA), Rhinovirus (RV) and Adenovirus (HdAV) are the most common viruses.[9] However, coinfection and secondary infection are common. The primary infection can predispose to secondary bacterial infections such as *Streptoccocus pneumonia*, *Haemophilus Influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumonia.*[10] In developing countries where resources are limited the ARTIs are treated empirically with antibiotics based on suspicion of bacterial infection which indeed could be one of the major causes of high morbidity and mortality.[11] Thus, efficient use of the viral culture, rapid antigen test and recent advances in molecular technology can be useful, such as multiplex PCR which offers rapid, sensitive, and simultaneous detection of several viruses and co-infection in the clinical samples.[12,13]

The respiratory virus causes a great burden of disease and more than one-third of death among children less than 5 years is due to respiratory disease. Here, we carried out a retrospective study on archived left-over samples from Influenza surveillance to identify the etiology of ARTI among children below 2 years in 2019, using a multiplex PCR assay.

MATERIALS AND METHODS

We used a retrospective cross-sectional design using the left-over samples from the Influenza surveillance program at the National Influenza Centre (NIC) from Jan-Dec 2019. This study was approved by the Nepal Health Research Council (815-2020, Dec). Only samples from children of 2 years or below were included in the sampling frame. The systematic random sampling method was used to select samples to ensure temporal distribution. We attempted to sample the first 10 samples with adequate volume (at least 300µL) and quality from each month.

RNA was extracted using Qiagen Viral RNA Mini kit and subjected to Influenza testing by CDC Influenza kit and for other respiratory viruses by FastTrack Diagnostic (FTD) Respiratory pathogens21 rt-PCR kit on ABI 7500 Fast Real-Time PCR platform following the manufacturer'sinstruction. Positive control, internal control, and negative control provided in the kit were run along with samples in each run. Amplification was carried out in 25 µL reaction volumes that include reverse transcription for 15 minutes at 50°C, denaturation for 1 minute at 94°C followed by 40 cycles of 8 seconds at 94°C and 1 minute at 60°C. The result was interpreted based on the manufacturer's interpretation criteria.

FTD respiratory Diagnostic (FTD) respiratory pathogens 21 PCR kit uses for the detection of Influenza A, Influenza A (H1N1) swl, influenza B, Corona viruses NL63, 229E, OC43 & HKU1, parainfluenza 1, 2, 3 & 4, human metapneumovirus A & B (HMPVA/B), rhinovirus, respiratory syncytial viruses A & B (RSVA/B), Adenovirus (HAdV), enterovirus, parechovirus (HPeV), bocavirus, *Mycoplasma pneumoniae,* including internal control.

RESULTS

Eighty samples that were sufficient in quantity and of high quality were processed. Of the study population 50 (62.5%) were male; male and female ratio is 1.66. They were all aged less than 2 years, 80% were ≤ 1 year. Among 80 samples, 46 (57.5%) were positive of which most common was Respiratory Syncytial Virus A/B: 13(16.25%), followed by Adenovirus: 12(15%), Influenza A: 9(11.25%), M. Pneumonia: 4(5%), Parainfluenza Virus-3: 4(5%), Human Metapneumovirus Virus A/B, Human parechovirus, Rhinovirus, Influenza B: 3(3.75%), Para Influenza virus 4, Para Influenza Virus 2, Para Influenza virus 1 & Coronavirus 43: 1(1.25%) (Table 2). Co-infection was detected in 11 samples (13.75%) of total tested samples and in 7 samples (63.63%) viral co-infection was observed involving mainly HRSVA/B. The positivity of ARTI cases showed mainly in October & November with concentrated cases of HRSVA/B.

Table 1: Distribution of samples month wise & positivity

Month	Number of samples processed	Positive N (%)
January	10	7(70%)
February	8	2(25%)
March	5	4(80%)
April	8	4(50%)
May	7	4(57.14%)
June	6	2(33.33%)
July	8	6(75%)
August	0	0(0%)
September	8	3(37.5%)
October	10	8(80%)
November	10	6(60%)
December	0	0(0%)
Total	**80**	**46**

Table 2: Distribution of etiology in 80 samples

Etiology	N(%)
Influenza A*	9(11.25)
Influenza B *	3(3.75)
HRSVA/B	13(16.25)
Rhinovirus	3(3.75)
Adenovirus	12(15)
HPIV-1	1(1.25)
HPIV-2	1(1.25)
HPIV-3	4(5)
HPIV-4	1(1.25)
HMPVA/B	3(3.75)
Mycoplasma Peumoniae	4(5)
HPeV	3(3.75)
Cor43	1(1.25)
Coinfection	11(13.75)
Total positive cases	**46(57.5)**

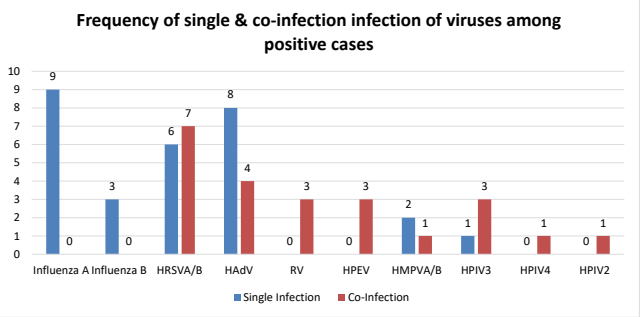

Figure 1: *Frequency of single and coinfection of viruses among positive cases*

DISCUSSION

World Health Organization (WHO) has identified ARI as the fourth leading cause of mortality worldwide, particularly in children, are mostly caused by respiratory viruses.[14] Human parainfluenza viruses 1, 2, and 3 (HPIV1-3), the respiratory syncytial virus (RSV), the influenza A and B virus, the human metapneumovirus (hMPV), the coronavirus (CoV), the enteroviruses (EV), the adenovirus (AdV), and the human bocavirus (HBoV), which accounts for more than 60% of ARIs are among them.[14,15] Influenza, a respiratory virus that is highly contagious and causes respiratory infection, is a prominent viral disease that has caused significant mortality and morbidity worldwide, mainly in young children.[16] Each year, influenza outbreaks are thought to affect 5-10% of adults and 20-30% of children worldwide, resulting in 3-5 million cases of severe illness and 290,000–650,000 fatalities.[17,18] RSV-associated lower respiratory tract infections caused 3.6 million hospitalizations and 26,300 mortality in children aged 0 to 60 months in 2019.[19] Similar to this, 5% to 10% of infant respiratory tract infections are caused by HAdV.[20]

In this study, RSV was detected in more than half (57.5%), followed by the HAdV and Influenza. Similar findings were reported ina study conducted in semi-urban Nepal Maria Mathisen et al[21], Broor et al[22] (India), and Yu-Wen Chen et al[23] (Taiwan). The coinfection of RSVassociated with other viruses like HAdV, HPIV-3, RV & HPEV werefound, however no RSV coinfection with Influenza positive was found unlikely to the previous study Bishu et al[24] which shows RSV as the most frequently detected coinfection with influenza positive samples. Following RSV, HdAV and Influenza were the most common viruses in this study, as reported in Prabhakar et al[25] and Flora De conto et al[26]. We also identified Mycoplasma pneumoniae, Human metapneumovirus, HPeV& HPIV in the respiratory samples. The presence of these viruses is reported in this study.

There were several limitations; mainly the limitation of the study was the sample size due to limited archived samplesquantity left over& feasibility of reagent due to lockdown. In particular, the sample is not equally represented across all months, not able to pick any sample of August and December and thereby not enough to ascertain the significance distribution of the whole year scenario or any seasonal distribution of the viral etiology. This result doesn't show a direct association between viral or bacterial infection and any seasonal distribution. However, the result of this study provides evidence of the viruses and there is limited information on the etiology of ARI thus suggesting the need for community surveillance to enhance public health efforts and scientific understanding of respiratory infection for appropriate preventive, treatment & management strategies through strengthening molecular diagnostic methods which provide a rapid, sensitive and specific approach to improve the etiological diagnosis for ARI.

CONCLUSIONS

Although only a small sample size was used for the study, which was primarily intended to test for influenza, it demonstrates the presence of other viral pathogens that have been linked to pneumonia and serious illnesses like RSV, parainfluenza, metapneumovirus, rhinovirus, and HAdV, which are associated with a significant rate of morbidity, mortality, and hospital admission in the younger population. Thus, it is strongly advised to implement wide multiplex testing by enhancing and strengthening the sentinel site for patient treatment and disease transmission prevention at an evident time, both as a standard diagnostic test and as a component of a surveillance program.

Conflict of Interest- None

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