CHAPTER 3

RESULTS AND DISCUSSIONS ON SURVEILLANCE OF RESPIRATORY VIRUSES CIRCULATING IN EASTERN INDIA DURING 2009-2012
3.1. **Surveillance of common respiratory viruses circulating in Eastern India during 2009-2012**

3.1.1. **Introduction**

In developing countries acute respiratory infections (ARI) are a leading cause of morbidity and mortality in infants and children. According to the World Health Organization (WHO), ARI accounts for approximately 20% of all deaths among children under 5 years [WHO, 2010], and 70% of these deaths occur in Africa and Southeast Asia [Williams et al., 2002]. Furthermore, viruses are leading causes of ARIs [Armstrong et al., 1999]. In children, respiratory syncytial virus and influenza viruses induce bronchiolitis, asthma exacerbation and pneumonia, leading to high rates of hospitalization [El-Hajje et al., 2008; Di Carlo et al., 2009]. Human rhinovirus, believed previously to cause only mild upper respiratory illnesses, has also been found in association with acute and chronic lower respiratory tract infections, including asthma exacerbations and chronic obstructive pulmonary disease (COPD); although the role of human rhinovirus as a cause has only been established for asthma [Papadopoulos et al., 2002; Friedlander and Busse, 2005; Xatzipsalti et al., 2005; Pierangeli et al., 2007; Singh and Busse, 2007; Matthew et al., 2009]. It was reported that metapneumovirus accounted for approximately 5–10% of all acute respiratory infections in children and adults [van den Hoogen et al., 2001]. In addition, infections caused by other respiratory viruses such as parainfluenza viruses and coronaviruses occur worldwide. Most studies based on laboratory diagnosis in hospitals are still restricted to influenza and respiratory syncytial virus. On the other hand, emerging respiratory viruses have been a subject of concern because of the risk of rapid spread and high fatality rates due to lack of both diagnosis and effective antiviral therapy. In eastern India, frequency and genetic diversity of influenza viruses during 2005–2009 have been reported [Agrawal et al., 2010], but no information was available from the Indian subcontinent regarding other respiratory viruses. The present study attempted to identify common circulating respiratory viruses in addition to influenza during 2009 through 2012 in the eastern region of India.
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3.1.2. Patient enrolment, collection and sampling strategy

3.1.2.1. Patient Selection criteria

Patient selection criteria include fever (>38°C) and two or more symptoms of ILI (cold/cough, sore throat, myalgia, body ache) according to WHO guidelines.

3.1.2.2. Collection and transport of samples

Nasal and/or throat swabs were collected from 7606 patients from the outdoor patient ward of B. C. Roy Memorial Hospital for children (BCRMHC), Kolkata, India, Nilratan Sarkar Medical College and hospital (NRS), Kolkata, India, National Medical College, Kolkata, India and R G Kar Medical College and Hospital (RGKMCH), Kolkata, India, from January 2009 to December 2012. The BCRMHC is one of the largest children hospitals in Eastern India, treating patients from rural and urban areas located in and around (up to 80 km) of Kolkata. Specimens in the form of sterile viscose swabs were transported in viral transport media (VTM- Hanks balance salt solution, Penicillin-Streptomycin and 2% BSA) to the laboratory. Three aliquots were made and stored at -80°C for further use.

3.1.2.3. Viral RNA isolation and virus culture

RNA was extracted from 200 µl clinical sample using commercially available QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) as per manufacturer’s instructions and stored at -80°C for subsequent RT-PCR assays. Cells were cultured in MEM (supplemented with 10% fetal bovine serum and antibiotics) and incubated at 37°C with 5% CO₂ (Shih et. al. 1999). 100-200 µl of Specimens were inoculated onto 5x10⁵ MDCK cell culture to isolate Influenza virus (Figure 3.1.). These cells were examined daily for cytopathic effect for 2-4 days. Hemagglutination (HA) test was performed on cell culture supernatant to estimate influenza virus titer.
3.1.3. Screening of clinical sample

From January 2009 through December 2012, 7606 samples from patients suffering with influenza like illness were tested; Out of these 7606 samples 49.4% patients were of less than 5 years of age. Remaining 50.6% patients were from age groups 5-15 years (14.2%), 15-35 years (19.4%), 35-45 years (6.1%), 45-60 years (7.3%) and >60 years (3.7%) respectively. Out of 7606 samples screened, 2359 (31.01%) were positive for one or more respiratory viruses; of which 22.8% were positive for Inf A which comprised of both influenza A(H1N1)pdm09 (14.7%) and Inf A(H3N2) (8.1%), Inf B was detected in 21.1% cases, Inf C (0.2%), PIV 1 (0.68%), PIV 2 (0.55%), PIV 3 (1.53%), and PIV 4 (0.64%), RSV (16.45%), HMPV (3.94%), and HRV was found in 0.17% cases (Figure 3.2.). During the study period, 121 samples were observed to be co-infected with RSV and Inf B viruses. Furthermore HMPV was observed along with RSV (n=19), Inf A (n=2) and Inf B (n=3) viruses. Mixed infection of Inf A and B was detected in 14 cases.
3.1.4. Correlation of virus infection with the age and gender distribution

Among the 7606 patients tested during the study, 4904 (64.5%) were males and 2702 (35.5%) were females (ratio M/F = 1.81). Based on data, 64.5% (% positive=4904/7606 X100) positive cases were male and 35.5% (% positive=2702/7606 X 100) were female. No gender specificity of infection was observed. Although the age of patients ranged from 1 month to >60 years, infections due to Inf A (H3N2), Inf B, HMPV and RSV were high among children under 5 years of age followed by 5-15 years and 15-35 years of age; whereas, adults (15-35 years of age) and elderly people were mainly infected with influenza A(H1N1)pdm09 virus. Even though the frequency was very low but Inf C and HCoV infection was predominantly observed among the children under 5 years of age. Similarly higher percentage of PIV 1 infection was observed among children of early age group (<5 years) followed by 15-35 years of age. In other age groups PIV 1 was not observed. The elderly people (>60 years of age) were at high risk for PIV 2 and PIV 4 infection; followed by the patients of 45-60 and <5 years of age group. Whereas, the infection rate for PIV 3 was high among the patients <5 years of age group. For HRV infection, the risk group was 35-45 years followed by 45-60 years of age. During this study, all mixed infections were observed only in children aged less than 5 years. The frequency of viral infection in different age groups has been shown in Figure 3.3.

Figure 3.2. Percent positivity of human respiratory viruses circulating in eastern India during the study period.
Figure 3.3. Age wise distribution of patients infected with different human respiratory viruses during the study period (2009-2012).

3.1.5. Respiratory virus infection with distinct meteorological conditions

The respiratory virus infection in eastern India from 2009 through 2012 with different meteorological variants is shown in Figure 3.4. In eastern India it was observed that upto April 2009 only HMPV circulated in this region; influenza A virus appeared during May 2009 and became predominant. During May-July only influenza A circulated in this region. During August HMPV infection was also detected. Influenza A, HMPV, PIV-1 and 2 infections was observed between September and October. In November 2009 circulation of influenza A, influenza B, RSV, PIV-1 and 2 was observed. During December 2009 influenza A and influenza B viruses circulated in this region although the rate of influenza B infection was very less. During February 2010-December 2010 influenza B, RSV, HMPV circulated in eastern India. From July 2010-October 2010 influenza A also reappeared. In case of parainfluenza viruses PIV-2 infection was first observed during June and was detected in rest of the year. PIV-3 and 4 infections were first detected during August; whereas PIV-1 infection was first observed during September. In the year 2011 influenza A infection showed a peak in June and disappeared after October and from October onwards influenza B and RSV infection occurred predominantly; although HMPV infection was observed with a moderate rate. PIV-1-4 infection was also detected throughout the
year except in January and February. In the first half of 2012 RSV was predominant however in later half influenza B and PIVS were also observed. Correlation of rainfall with prevalence of virus infections was observed only for influenza A.

Figure 3.4. Correlation of meteorological variations with prevalence of Influenza A, Influenza B, Influenza C, RSV, HMPV and HPIV [1-4] during 2009–2012 in eastern India.

3.1.6. Discussion

In 2004, surveillance for influenza viruses was expanded to India, as part of the global influenza surveillance network. However, specific diagnosis which requires laboratory tests, were not widely available in eastern India. Hence, the information on epidemiology and clinical features of respiratory virus infection in India is based entirely on research studies and the disease burden or seasonal prevalence of respiratory viruses remains largely undefined. This study attempted to complete the information on circulating respiratory viruses among patients attending the outpatients departments of different hospitals with acute respiratory infections in the eastern region of India during 2009 through 2012. During this surveillance, influenza A infection was found to be most prevalent followed by influenza B. This was consistent with the previous report from eastern India, where higher prevalence of influenza A was observed compared to influenza
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B (Agrawal et al. 2010). During 2010 increased frequency of influenza B infection was observed this could be due to the post-pandemic effect of influenza A(H1N1)pdm09 following which seasonal influenza A/H1N1 and influenza A/H3N2 viruses disappeared during 2009–2010. This is consistent with studies from Vietnam (Do et al. 2011) and other Asian countries (Mathisen et al. 2010). Metapneumovirus has been associated with both upper and lower respiratory tract infections in children (3–13%) (van den Hoogen et al. 2001; Bosis et al. 2005; Gerna et al. 2005; Choi et al. 2006; Sarasini et al. 2006; Boivin et al. 2007). During this study period, metapneumovirus was found in only 3% samples. This finding corroborates with reports from Canada, England and the USA, where the infection rate due to metapneumovirus varies from 2% to 4.5% (Stockton et al. 2002; Falsey et al. 2003; Boivin et al. 2004), but is significantly less compared to the prevalence in Netherlands (10%), Australia (9.7%), and Chile (5.4%) (van den Hoogen et al. 2001; Luchsinger et al. 2005; Mackay et al. 2006). Respiratory syncytial virus which is the most common etiological agent of viral lower respiratory tract infections in infants and young children in the world caused 33.8 million new episodes in children <5 years of age (Nair et al. 2010) and is also associated with hospital admission of 3–9 per 1,000 children below 1 year (Pancer et al. 2011). In Thailand alone, 417.1/100,000 incidences of pneumonia per year are attributed to respiratory syncytial virus (Olsen et al., 2010). Worldwide, respiratory syncytial virus has been identified as the cause of 3.4 million cases of acute lower respiratory infections requiring hospital admission. Among all age groups, 7.1% cases were found positive for respiratory syncytial virus during 2010–2011 in this study. Following respiratory syncytial virus, parainfluenza virus-2 was the most predominant virus (6%) compared to parainfluenza virus-1, - 3 or -4. Consistent with surveillance results during 2006–2009, in eastern India (Agrawal et al. 2010; Roy et al. 2011) and in Bangladesh (Zaman et al. 2009) children under 5 years old were found to be most vulnerable to infections due to influenza B and influenza A (H3N2) viruses. Whereas, influenza A(H1N1)pdm09 affected adults and the elderly which is consistent with findings from other studies (Cauchemez et al. 2009; John and Moorthy 2010; Mukherjee et al. 2010). Similar to influenza viruses, metapneumovirus infection was also detected mainly in children (less than 5 year of age), suggesting early acquisition of infection (van den Hoogen et al. 2001; Lu et al. 2011; Zappa et al. 2011). During 2007–2008, only 8.7% of respiratory syncytial virus infection was found among children (Agrawal et al. 2009b); whereas in the same
geographical region, 13.7% samples were found to be positive for respiratory syncytial virus in children (under 5 years) during this study period (2010–2011). The higher prevalence of respiratory syncytial virus during this study period could be due to lower activity of influenza A viruses compared to the activity of influenza A viruses in 2007–2008 (Agrawal et al. 2009b). Among children with acute respiratory infections, co-infection with one or more respiratory viruses has been observed (Bonzel et al. 2008; Canducci et al. 2008) which correlates with results of present study, where predominant mixed infections were observed only in children. Unlike temperate countries where the prevalence of seasonal influenza may reach epidemic proportions during the winter months (John and Moorthy 2010), in tropical countries like India, year round circulation of strains has been reported, though the infection peaks during rainy season (June–September) (Agrawal et al. 2009a; 2010). There could be several factors such as socio-economic, environmental, education, overcrowding and other factors, which could affect the seasonal incidence and distribution of viral infections. In eastern India, influenza B was found to be prevalent after the monsoon season as well as in the winter months (Roy et al. 2011), and influenza A viruses predominated during the monsoon (June–July) (Agrawal et al. 2009a; Mukherjee et al. 2010). During 2010–2011 seasonal infection due to influenza A [influenza A(H1N1)pdm09 or influenza A/H3N2] correlated with previous reports. However the seasonal pattern of infection with respiratory syncytial virus during 2010–2011 was found to be different from the previous reports from Kolkata, India (Agrawal et al. 2009a; b) and Bangladesh (Huq et al. 1990), where respiratory syncytial virus infection was found more commonly in the winter months or the dry seasons. Such variations in seasonal incidence are difficult to explain, as virus infection could be affected by a large number of different factors (Weber et al. 1998). Previous studies in temperate climates showed that parainfluenza virus-1 and -2 infection occurs annually (Laurichesse et al. 1999; Karron and Collins 2007), parainfluenza virus-4 infection occurs twice-yearly during the late fall and winter (Aguilar et al. 2000; Vachon et al., 2006; Lau et al. 2009) and parainfluenza virus-3 infection occurs mainly during late spring and summer (Laurichesse et al. 1999). These differences may be attributed to different geographical regions and study years. However, as the numbers of positive cases were very low in the present study, the seasonal prevalence of influenza C, parainfluenza viruses and human rhinovirus could not be determined.
Therefore, large-scale studies over a broader geographical range and longer time period may help to understand the seasonal infection pattern of respiratory viruses in India.