Virological surveillance of influenza virus in acute respiratory infection

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Abstract
Introduction: Influenza is viral fever with the main symptom of respiratory tract like cough, fever, breathlessness, nasal discharge, sore throat. The influenza affects a large segment of the world population resulting in significant mortality, morbidity and economic loss.
Objective: To monitor the trend of influenza A virus. To know the prevalence of influenza A, H1N1 and seasonal H3 subtypes of influenza virus. To study the seasonal pattern of influenza A virus.
Materials and Methods: This prospective study was carried out in the department of Microbiology, B.J. Medical College, Ahmadabad from January 2014 to December 2014. Surveillance samples were taken as per ILI (Influenza like illness) case definition for out Patient department and SAR (sever acute respiratory syndrome) for indoor patients. Total 614 samples are taken and tested with PCR method.
Result: Out of 614 samples total 201(32.73%) were positive for influenza. Out of 201 positive samples, 13 (2.11%) were positive for swine H1N1 and 11 (1.79%) for seasonal H3, 177 (28.82%) were positive for influenza A.
Conclusion: Prevalence of influenza A was common in winter and seasonal H3 was common in late monsoon. Swine H1N1 and seasonal H3 both are more common in male than female. Swine H1N1 is more in >12 age and seasonal H3 is more common in <12 age.

Keywords: Influenza A, Swine H1N1, Seasonal H3, Fever, cough, Breathlessness.

Introduction
Respiratory illnesses due to influenza virus infection are a major cause of morbidity and mortality worldwide. Lower respiratory tract infections are a leading cause of deaths in developing countries and are also the third leading cause of death worldwide. Globally influenza is responsible for 250,000 to 500,000 deaths annually. In April 2009, the first case of influenza A H1N1 was reported from Mexico. It was a reassortant between previously circulating swine virus and a Eurasian swine virus and was also called swine origin influenza.

(S-OIV). It spread from person to person and caused a pandemic. Subsequently, the infection led to spread of disease across 74 countries with 30,000 confirmed cases on June 11, 2009. Total of 214 countries were affected by the pandemic worldwide.

In India, the first case of influenza A H1N1 was reported on May 16, 2009 from Hyderabad. The World Health Organization declared the post pandemic phase on August 10, 2010. The Central and State governments in India had taken the pandemic very seriously and made several unprecedented and innovative interventions including the pandemic preparedness plan. Of all the viruses that cause respiratory illness, influenza virus plays a dominant role. Influenza viruses are known to cause frequent epidemics and periodic pandemics, and are unique with regard to their antigenic variability, Seasonality and impact on general population. Though children are mainly affected during epidemics, the viruses are also responsible for substantial mortality in the aged and chronically ill persons. Polymerase chain reaction (PCR) is a technique used to amplify specific regions of DNA from very low levels of starting template DNA. Reverse-transcription PCR (RT-PCR) is an extension of this technique in which template RNA is first reverse-transcribed into complementary DNA (cDNA). This cDNA then undergoes amplification by PCR. Vaccines and antiviral drugs are the two primary methods of implementing influenza prophylaxis.

Materials and Methods
This prospective study was carried out in the department of Microbiology, B.J. Medical College, Ahmadabad from January 2014 to December 2014. Surveillance samples were taken as per ILI (Influenza like illness) case definition for out Patient department and SAR (sever acute respiratory syndrome) for indoor patients. Total 614 samples suspected of fever, cough, sore throat, nasal catarrh, shortness of breath were included in this study. Two samples (one nasal and one oropharyngeal/throat swab) were collected in VTM from the suspected patients of Influenza. Samples were labeled and triple layer packing was done and sample along with request form were transported in cold chain to Virology laboratory (Microbiology department). Sample was proceeding in...
BSC class II. RNA extraction was done by using QIAamp Viral RNA Kit. First RNA extraction is done using all above listed items. master mix and 5μl of controls were taken, put the plate and process the sample in PCR machine. Thermo cycler raises and lowers the temperature to permit and regulate three steps of PCR (DNA denaturation step, primer anneling step, extension reaction step). In the PCR machine following cycles are automatically done.

**Holding Stage**
1. Step-1 50.0°C for 30 minutes.
2. Step-2 95.0°C for 10 minutes.

**Cycling Stage (45 cycles)**
1. Step -1 95.0°C for 00.15 second
2. Step-2 55.0°C for 00.30 second.

The whole procedure is repeated in a programmable thermal cycler. Generally, 30-50 thermal cyclers produce millions of copies of the chosen section of the target organism DNA.

After cycles are over PCR will be automatically stop, and we achieve a amplification graph on computer which is attached with machine. Ct value of each sample was detected and if the graph is raised, than consider it as positive. If not, than negative.

**Result**
Out of 614 total samples, Total 201 (32.73%) were positive for Influenza. Out of 201 positive samples, 13 (2.11%) were for Swine H1N1 and 11 (1.79%) for seasonal H3, 177 (28.82%) were positive for Influenza A. both Swine H1N1 and seasonal H3 are more common in male than female. swine H1N1 is more in >12 age and seasonal H3 is more common in <12 age. swine H1N1 is mostly occurs in winter and seasonal H3 is more common in late monsoon. (Fig. 1 to 3)

Fig. 1: Gender wise distribution of positive swine H1N1 and seasonal H3 case

The probable reason for predilection of male sex may be due to greater mobility, susceptibility and exposure to infection and also this age group consists of economically productive mobile population, travelling more for various reasons and most susceptible to
exposure to infection, so they get exposed to virus easily and get infected easily.\textsuperscript{11}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Age wise distribution of positive swine H1N1 and seasonal H3 case}
\end{figure}

Swine H1N1 prevalence is high in elder people may be due to immunity is tapering at this age and so this people are more prone to infection.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Month wise distribution of positive swine H1N1 and seasonal H3 case.}
\end{figure}

Prevalence of swine H1N1 in winter mainly because of this virus can survive and multiply more in cold & dry environment. Seasonal H3 also generally occur in winter but here it may be due to some mutation, it occur in late monsoon. The reason for monsoon Predilection might be due to climatic factors such as relative humidity, rainfall and differences in temperature may cause the rapid shedding of this virus.\textsuperscript{13}

\section*{Discussion}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Study} & \textbf{Positivity of Swine H1N1 (%)} & \textbf{Positivity for seasonal H3 (%)} \\
\hline
Our study & 2.11\% & 1.79\% \\
B. L. Rao, K. Banerjee et al, In Pune, India, 1978-1990.\textsuperscript{3} & 0.9\% & 1.16\% \\
\hline
\end{tabular}
\caption{Comparison of our study with other influenza surveillance studies}
\end{table}

In our study positivity of Swine H1N1 (2.11\%) is comparable with the study of B.L. Rao et al. Difference in positivity with B.L. Rao et al might be due to difference in strain and different in geographical area. Seasonal H3 positivity in our study (1.79\%) is well correlated with the study of B.L. Rao et al.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Study} & \textbf{Male\%} & \textbf{Female\%} \\
\hline
Our study & 53.84\% & 46.15\% \\
Dr. Tripurari Kumar et al, Punjab,\textsuperscript{9} & 54.1\% & 45.9\% \\
\hline
\end{tabular}
\caption{Ratio of male and female in positive Swine H1N1 patients}
\end{table}
In our study prevalence of Swine H1N1 is more in male compared to female which is well correlated with study of Dr. Tripurari Kumar, et al.

<table>
<thead>
<tr>
<th>Study</th>
<th>Male%</th>
<th>Female%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our study</td>
<td>63.63%</td>
<td>36.36%</td>
</tr>
<tr>
<td>Susanna Esposito et al.</td>
<td>60.1%</td>
<td>39.9%</td>
</tr>
</tbody>
</table>

Table 3: Ratio of male and female in seasonal H3 Positive patients

In our study prevalence of seasonal H3 is more in male compared to female which is well correlated with study of Susanna Esposito et al.

Our study shows that prevalence of Swine H1N1 in pediatric age group (23.07%) is lowest, followed by 12-40 years age group (38.46%), followed by aged >40 years (38.46%). The study of Arvind Chandora et al also shows lowest prevalence in 0-20 years and nearly equal prevalence in middle age and > 40 years of age group. Difference in the prevalence in pediatric age group is due to selection of different age group in the study. Study done by M. P. Tambe et al shows similar prevalence (23.94%) in (<10 year) pediatric patients.

In our study prevalence of seasonal H3 is highest in <12 years age group (54.54%) followed by middle age group (45.45%) and no sample positive in age group >40 years. The study done by Sam Peter et al in Kerala shows relatively higher prevalence in middle age group (47.91%) than pediatric age group (43.75%) and lowest prevalence in > 45 years of age group (8.33%). This might be due to difference in geographical area and differences in sample size selected.

In our study, Fig. 3 shows prevalence of Swine H1N1 is highest in December month (38.46%). The study done by Archana Choudhry et al in 2009 in Delhi (29.45%) shows highest no. of cases in December month. This was an alarming signal as we faced a big epidemic of H1N1 in 1st quarter of 2015. Seasonal H3 shows maximum number of cases during August, followed by September, followed by October month. No cases detected in other months of the year.

**Conclusion**

Influenza infections cause substantial morbidity and mortality every year. Influenza surveillance has focused on virological monitoring and collection of specimens to guide vaccine strain selection. Continuous monitoring would be required for early detection of any antigenic variants to understand the seasonality and analyses factors such as temperature and rainfall in the transmission of influenza viruses. Implementation of a sentinel network reinforces the importance of a national strategy for the control and prevention of Influenza.

**References**
