

First Dominant Circulation of Influenza A/H3 among Patients with Acute Respiratory Tract Infections in a Tertiary Care Hospital in Dhaka 2022

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Abstract

The seasonal circulation of influenza viruses varies from year to year. We have retrospectively analyzed our hospital data of respiratory pathogens among patients of respiratory tract infections and extracted circulation of influenza viruses and characterized patients with influenza attended to a tertiary care hospital in Dhaka from July 2021 to September 2022.

We have analyzed data of real-time reverse transcription polymerase chain reaction testing of respiratory panel done in QIAstatDx (Qiagen, Germany) using nasopharyngeal samples to identify the presence of influenza viruses and to determine the subtypes.

A total of 547 patient's data were analyzed in this study, with an influenza positivity rate of 15.62% in 2021 and 23.63% in 2022. We identified an exclusive circulation of influenza A/H3 viruses in 2022 while co-circulation of Influenza A (H1N1 pdm 09 – 10.15%, H3– 2.34%, A not subtyped – 2.34%) and influenza B (0.78%) was observed in 2021 in our cohort. Further, subtyping data showed that both Influenza A/H1N1 pdm09 and Influenza A/H3 was equally observed in influenza like illness (ILI) and severe acute respiratory infection (SARI) patients.

This small study in our cohort warrants further elaborate investigation to know circulating influenza A subtyping in the country which may assist health care providers in making treatment decisions and hence, appropriate patient management.

Key words: Flu-like symptoms, Influenza, multiplex PCR, Dhaka



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Introduction

Influenza is an acute respiratory disease that cause global epidemics and pandemics in humans with significant

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mortality and morbidity reported every season¹. The CDC, USA documented that seasonal influenza was responsible for 24,000-62,000 deaths during 2019-2020 season². Influenza remains as the most common viral infections affecting 5%-15% of global population resulting in an estimated deaths of 250 000 to 500 000 each year^{3,4}.

Three distinct immunologic types of influenza virus A, B and C have been classified⁵. Influenza is an RNA virus of Orthomyxoviridae family. The three major pandemics of the twentieth century were caused by influenza virus type A. Influenza A viruses are classified into 18 haemagglutinin (H1-H18) and 11 neuraminidase (N1- N11) transmembrane proteins based on the antigenic properties of these glycoproteins⁶. However, three main categories – H1 (H1N1

pdm09 & H1N1 seasonal - two common strain of H1N1), H3 & H5 are mostly circulating in the community. Epidemics are mostly caused by influenza virus type A. Type A virus can infect a variety of different host species including man, birds, pigs, horses and other animals. Influenza type B and C viruses only infect humans; however, influenza type C virus has been isolated in pigs⁷ and dogs⁸.

The pandemics of influenza are typically due to the emergence of subtypes of influenza A viruses as influenza B and C viruses have not been known to cause pandemic in the past⁹. When a significant “antigenic” shift in at least one of the influenza A virus surface proteins haemagglutinin and neuraminidase occurs spontaneously, there may be no immunity to this new strain. And if the virus also achieved effective human to human transmission and replication this can lead to a pandemic. It happened with Spanish flu caused by influenza virus of the A/H1N1 subtype in 1918^{10,11}. Further, evidence has shown that multiple lineage of the same virus subtype can co-circulate, persist and re-assort in epidemiologically significant ways¹². The predisposition of influenza virus to undergo frequent and permanent antigenic changes necessitates constant monitoring of the global situation and annual adjustments in the composition of influenza vaccines¹³.

In Bangladesh influenza is found all through the year and its annual seasonal epidemic occurs in monsoon period, from May to September¹⁴. Laboratory diagnosis of influenza virus, especially at the beginning of a new community outbreak has important implications for case management, such as infection control procedures, consideration of antiviral treatment options and avoiding the inappropriate use of antibiotics. To identify and explore the circulating predominant Influenza subtype we analyzed data of multiplex real time PCR from nasopharyngeal swabs from patients with acute respiratory tract infections attended in Evercare Hospital Dhaka during July 2021 to September 2022.

Material and Methods

Ethical statement

This study proposal was approved by the Research and Ethics Committee of Evercare Hospital Dhaka (ERC 25/22-1). This study was exempt from obtaining participant’s consent since it is a data based retrospective study. All samples were de-identified to protect patients’ private information.

Patient and clinical specimen

The data of the patients were taken from hospital information system of Evercare Hospital Dhaka, Bangladesh and the study period was between July 2021 & September 2022 irrespective of their age group. Nasopharyngeal swab samples were collected from all patients presenting suspected flu like symptoms by trained medical personnel in virus transport medium (Biocomma, China) and were transported to molecular laboratory by patient care attendant (PCA). Clinical correlation was done among ILI (Influenza like illness) and SARI (Severe acute respiratory illness) group. Though the clinical symptoms of both groups are almost similar, ILI and SARI were separated based on WHO guideline (ILI define patients’ fever with respiratory symptoms and SARI define patients’ fever with respiratory symptoms who requires hospitalization).

Diagnosis of Influenza types & Influenza A subtyping

For diagnosis of influenza, its type and subtyping, specimen containing nasopharyngeal swab stick was inserted into a Virus Transport Medium (Biocomma, China) and the samples were tested soon after collection, remaining were refrigerated for up to 2-3 days as per laboratory policy. The test was performed in a QIAstat-Dx Analyzer 1.0 with QIAstat-Dx Respiratory panel (691214 CE-Marked) cartridge according to the manufacturer’s instruction (QIAGEN, 2020). A transfer pipette was used for dispensing transport medium liquid sample and 300 μ l of sample was transferred into the main port of the QIAstat-Dx Respiratory Panel Cartridge. The extraction, amplification, and detection of nucleic acids in the sample were performed automatically by the QIAstat-Dx Analyzer 1.0 and detected result were displayed in the monitor. Influenza A, Influenza B, Influenza A subtype H1N1 pdm09, Influenza A subtype H1 (H1N1 seasonal influenza before 2009) & Influenza A subtype H3 were detected and differentiated simultaneously with this cartridge. MS2 bacteriophage was used as internal control in this assay. A positive signal for the Internal Control indicates that all processing steps performed by the QIAstat-Dx Respiratory Panel Cartridge were successful and a negative signal of the Internal Control indicate the test should be repeated of tested and positive patients with influenza virus infection

Table 1. Age distribution of study population and positivity rate

Age group (years)	Samples tested (n= 547)		Found positive (n= 119)		No. of sample tested/ no. of pos (%pos)
	ILI	SARI	ILI	SARI	
0 - 5	18	31	3	7	49/10 (20.4)
6 - 17	36	24	5	6	60/11 (18.3)
18 - 30	44	12	14	2	56/16 (28.6)
31 - 40	57	9	19	3	66/22 (33.3)
41 - 50	43	16	10	2	59/12 (20.3)
51 - 64	74	43	12	9	117/21 (17.9)
>64	46	94	11	16	140/27 (19.2)
Total	318	229	74	45	547/119 (21.8)

Clinical characteristics:

All distribution are showing in Table 1, and found distribution over all age group with predominance of 31-40 age group.

Patients presented with flu like symptoms particularly with fever along with cough, sore throat, rhinitis, headache, difficulty in breathing, body ache were the main clinical features of this study group. Gastrointestinal features were also found, such as diarrhea and/or vomiting in some cases. Fever was present in all ILI and SARI cases.

Among influenza cases of ILI patients, majority of influenza positive patients had cough (97.3%) and cold/runny nose (77%) followed by other features. Among SARI Influenza positive cases, breathing difficulty (77.3%) was noticeable feature along with cough (95.6%) and runny nose (66.7%). (Table 2).

Circulating strain and seasonality

A total of 119 (21.8%) cases were found Influenza positive by cartridge-based RT-PCR test among 547 flu like symptomatic patients where influenza positive ILI cases were 74 (23.3%, out of 317) and SARI were 45 (19.6%, out of 230). Influenza A positive cases were predominant (95%) in 2021 and only single case (5%) of influenza B was found in 2021. Out of 128 samples in 2021 Influenza A was found positive in 19 (14.84%) samples and out of 419 samples in 2022 Influenza A was found in 99 (23.63%) samples. We did subtype of total 118 influenza A positive cases to determine the strain of Influenza A/H1 (H1N1 seasonal influenza before 2009), Influenza pandemic strain A/H1N1 pdm09, and Influenza A/H3. Subtyping data showed that both Influenza A/H1N1 pdm09 and Influenza A/H3 was equally observed in ILI and SARI patients in 2021 (Table 3).

Table 2. Clinical symptoms of influenza and non-influenza cases

Symptoms	ILI		SARI	
	Inf +ve, no. (%)	Inf -ve, no. (%)	Inf +ve, no. (%)	Inf -ve, no. (%)
Fever	74 (100)	243 (100)	45 (100)	185 (100)
Cough	72 (97.3)	196 (80.6)	43 (95.6)	145 (78.4)
Cold/ Runny nose	57 (77)	185 (76.1)	30 (66.7)	103 (47.0)
Bodyache	20 (27)	35 (14.4)	11 (24.4)	19 (10.3)
Headache	24 (32.4)	42 (17.3)	9 (20)	23 (12.4)
Sore throat	11 (14.9)	65 (26.7)	6 (13.3)	9 (4.9)
Breathing difficulty	10 (13.5)	28 (11.5)	33 (73.3)	93 (50.3)
Vomiting	3 (4)	4 (1.6)	12 (26.7)	26 (14.0)
Diarrhea	2 (2.7)	1 (0.4)	5 (11.1)	13 (7.0)

Table 3. Proportion of ILI and SARI cases with influenza virus infection

	ILI, no. (%) n = 317	SARI, no. (%) n = 230	Total, no. (%) n = 547
Influenza positive	74 (23.3)	45 (19.6)	119 (21.8)
Types	n = 74	n = 45	n = 119
Influenza A	73 (98.6)	45 (100)	118 (99.2)
Influenza B	1 (1.4)	0	1 (0.8)
Subtypes	n = 73	n = 45	n = 118
Influenza A/H1	0	0	0
Influenza A/H1N1 pdm09	7 (9.6)	6 (13.3)	13 (11.0)
Influenza A/H3	61 (83.6)	38 (84.4)	97 (82.2)
Influenza A other subtypes	5 (6.8)	1 (2.2)	6 (5.0)

The influenza activity is seasonal in Bangladesh and strain changes season to season. We started influenza testing from first July in 2021 and found 20 influenza positive cases (19 influenza A and one influenza B) out of 128 samples tested till December. Out of 19 Influenza A positive cases in 2021 we found 14 positive cases in September and 5 in October. Subtyping of these 19 influenza A showed 13 were pandemic strain A/H1N1 pdm09, 3 were A/H3 and 3 could not be subtyped (Table 4). From November 2021 to May 2022, we did not find any influenza positive cases against samples tested. In June

2022, we found 41 (30.8%, out of 133) Influenza A positive cases of which almost all of them were Influenza A/H3 except one influenza A which could not be subtyped. Same scenario was observed in July 2022 where 56 (44.8%) were Influenza A positive out of 125 sample tested where all were Influenza A/H3 strain except 2 untyped cases. We did not find any Influenza A/H1N1 pdm09 strain in this peak season (June – July) of 2022. From this study population we observed Influenza A/H1N1 pdm09 strain was predominant in 2021 and the dominancy completely shifted to Influenza A/H3 in 2022 (Table 4/ Figure 1).

Table 4. Month wise distribution of tested and positive patients with influenza virus infection

Month	No. of samples tested (n = 547)	No. of positive found (n = 119)					Total (%) pos
		Inf. A/H1 (%)	Inf. A/H1N1 pdm09 (%)	Inf. A/H3 (%)	Inf. A other type (%)	Inf. B (%)	
Jul-21	10	-	-	-	-	-	-
Aug-21	10	-	-	-	-	-	-
Sep-21	34	-	12 (35.3)	0	2 (5.9)	1 (2.9)	15 (44.1)
Oct-21	22	-	1 (4.5)	3 (13.6)	1 (4.5)	0	5 (22.7)
Nov-21	24	-	-	-	-	-	-
Dec-21	28	-	-	-	-	-	-
Jan-22	24	-	-	-	-	-	-
Feb-22	35	-	-	-	-	-	-
Mar-22	15	-	-	-	-	-	-
Apr-22	5	-	-	-	-	-	-
May-22	23	-	-	-	-	-	-
Jun-22	133	-	-	40 (30)	1 (0.8)	-	41 (30.8)
Jul-22	125	-	-	54 (43.2)	2 (1.6)	-	56 (44.8)
Aug-22	23	-	-	01 (4.3)	-	-	01 (4.3)
Sep-22	36	-	-	01 (2.8)	-	-	01 (2.8)

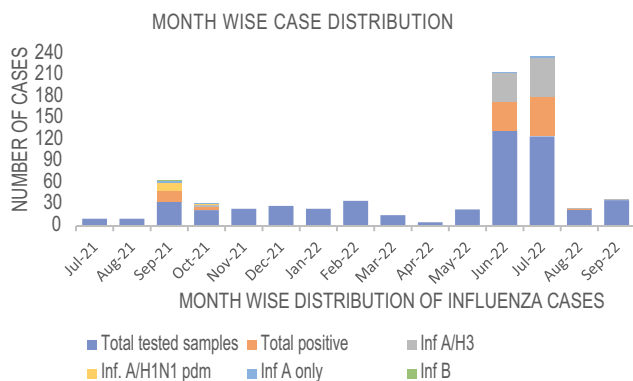


Figure 1. Distribution of Influenza cases/month in 2021 and 2022 among patients with respiratory tract infections attended in Evercare Hospital Dhaka

Discussion

In this study, influenza A virus was the most frequently identified within the study population in 2021 and 2022 season and there is only one subtype A/H3 found in 2022 and co-circulation of three subtypes with the dominance of subtype H1N1 pdm09 in 2021. Previous study reports show that both influenza A and influenza B virus was co-circulating in Bangladesh from 2010 with the dominance of influenza A except in 2016 when Influenza B was the dominating type¹⁵. Pandemic strain H1N1 pdm09 was the main influenza virus circulating in the country and this study shows only A/H3 is circulating in our cohort till date. National Influenza Surveillance, Bangladesh (NISB) data published in IEDCR website also support our findings¹⁶.

It is a matter of investigation whether COVID-19 pandemic influence the influenza epidemic. Recently published report showed that during the COVID-19 pandemic influenza epidemic in 2020 in Bangladesh started 18 weeks later, was 7.5 weeks shorter, and was less intense than the average epidemic of the four previous years. The 2020 influenza season started on the same week when COVID-19 control measures were halted, and 13 weeks after the measures were relaxed. In 2021 influenza detection continued low, however, season started at April with influenza B and switched to influenza A from September with huge dominance of H1N1 pdm09¹⁷. WHO updates in May 2022 also showed overall low influenza detection in Southern Asia during covid-19 pandemic¹⁸. Influenza A/H1N1 pdm09 activity decreased in Pakistan and sporadic influenza A/H3 virus detections were reported in India and Iran. In Southeast Asia, Malaysia continued to report detections of influenza A/H3 and B viruses. These reports support regional shifting of Influenza A subtype to A/H3 from H1N1 after the covid-19 pandemic.

We could not subtype 6 influenza A viruses among 118 influenza A positive cases. There might have mutation on primer binding sites or these may be new subtype because 18 haemagglutinin (H1-H18) and 11 neuraminidase (N1-N11) transmembrane proteins are existed and there is always chance of evolution of new subtype.

Previous study showed that anti-genic regions of the HA glycoprotein of A/H3 viruses evolve at a faster rate than A/H1N1 virus and influenza B virus antigenic regions¹⁹. These differences in rates of evolution lead to larger and more frequent A/H3 virus epidemics which could, in turn, drive evolution, resulting in people of a wide variety of ages becoming infected²⁰. A/H1N1 and influenza B viruses tend to cause smaller and less frequent epidemics and to infect younger individuals.

Appropriate treatment of patients with respiratory tract infection depends on accurate and timely diagnosis. Early diagnosis of influenza and its subtypes can reduce the inappropriate use of antibiotics and provide the option for proper antiviral therapy. Our findings will help to give an idea about circulating subtypes in the community. Large scale multicentric study is recommended to guide the policy makers for better explore, prioritize strategies for Influenza prevention and control in Bangladesh.

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