Diversity of Upper Respiratory Tract Pathogens in Patients having Flu-Like Symptoms during COVID-19 Pandemic in a Referral Hospital

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Abstract:

Background: Upper respiratory tract infections (URTIs) are caused by a wide range of viruses and bacteria, however, produce similar symptoms. Routine molecular tests are not performed and empirical use of antibiotics in treating URTIs is a major public health concern. In attempt to unveil the diversity of upper respiratory tract pathogens in the community during COVID-19 pandemic, we have screened 153 nasopharyngeal swab samples from patients having flu like symptoms.

Materials and methods: We tested nasopharyngeal swabs by real-time multiplex PCR for 19 viruses and 3 bacteria using cartridge based rapid PCR platform.

Results: Of 153 patients sample tested, 103 (67.32%) had a laboratory-confirmed respiratory pathogen. Of the 153 swabs tested rhinovirus/enterovirus was found 25(16.34%), influenza 18(11.77%), RSV 13(8.5%), SARS-CoV2-15(11.11%), other coronaviruses 11(7.19%), parainfluenza 16(10.45%), human metapneumovirus 8(5.23%). Out of 18 influenza cases influenza A was 17(11.12%) and influenza B was 1(0.65%). Among 17 influenza A viruses H1N1pdm09 strain was 9(5.88%), H3 was 5(3.27%). These data shows that even in COVID-19 pandemic period rhinovirus/ enterovirus and Influenza dominated over all other respiratory viruses and as a causative agent bacteria might play very insignificant role in URTIs.

Conclusion: Our data provides strong evidence against empiric antibiotic use for treating URTIs and highlights a strong need for improving the diagnostic capacity for URTIs by using more molecular testing in the country.

Key words: Respiratory pathogen, flu-like symptoms, multiplex PCR, COVID-19, Dhaka

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Introduction:

Acute respiratory tract infections (ARIs) are a primary cause of morbidity worldwide and a major cause of child mortality in the developing world (1). Mortality is mostly caused by pneumonia, a lower respiratory tract infection. On the other hand, upper respiratory tract infections (URTIs) cause huge morbidity in both child and adult. Though there are some reports exist about disease burden, socio-economic status, risk factor and causative agents for acute lower respiratory tract infections in children under 5 there is no study about detailed etiologic agents for URTIs in older children and adult (2-5). Diagnosing a particular respiratory viral infection based on the symptoms is difficult as all respiratory viral infections have overlapping symptoms. Worldwide data supports upper respiratory tract infections are principally caused by viruses and sometimes few bacteria (6,7). Demarcation of viral URTIs versus bacterial URTIs has the direct implication of antibiotics use. However, a lack of timely diagnosis by using rapid and accurate tests had contributed to overuse and abuse of antibiotics around the world (8). In Bangladesh, empirical use of antibiotics is very common due to a practice of self-medication and over-prescription by clinicians. This is particularly problematic for patients with URTIs, for which country lacks routine tests for viruses and bacteria. Common upper respiratory tract pathogens such as influenza, coronavirus, human rhinovirus (hRV), respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), adenovirus (ADV), human

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metapneumovirus (hMPV) and bacterial pathogens that are difficult to culture including Mycoplasma pneumoniae, *Chlamydophila pneumoniae* and *Bordetella pertussis* are generally not tested in Bangladesh.

Using multiplex real time PCR, we have screened 153 nasopharyngeal swab samples in this COVID-19 pandemic period from patients having flu like symptoms for coronaviruses including SARS-CoV-2, respiratory syncytial virus (RSV), rhinovirus, influenza virus with sub-types, parainfluenza virus 1-4, human metapneumoviruses, adenovirus, bocavirus and common three bacterial pathogen causing URTIs *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis* to know the pathogen paradigm existed in our cohort. Here we report that viral pathogens are mainly associated with flu like symptoms.

Materials and Methods

Ethical statement

This study proposal was approved by the Research and Ethics Committee of Evercare Hospital Dhaka (ERC 37/2022-05). This study was exempted from obtaining participant's consent since data extracted from hospital information system (HIS) for routine test and anonymization was done to protect patient's identity.

Patient and clinical specimen

Nasopharyngeal swab samples were collected for routine respiratory panel test targeting coronaviruses including SARS-CoV-2, respiratory syncytial virus (RSV), rhinovirus, influenza virus with sub-types, parainfluenza virus 1-4, human metapneumoviruses, adenovirus, bocavirus and common three bacterial pathogen causing URTIs *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis* from patients of any age group presenting flu like symptoms and were transported to laboratory.

The nasopharyngeal swabs were collected using the flock swabs (Biocomma, China) and stored in the Universal Transportation Medium (Chaoran, China) provided in the same collection kit. The samples were tested soon after collection and remaining were refrigerated for up to 2 -3 days as per laboratory policy.

Multiplex Real Time PCR

The QIAstat-Dx® Respiratory Panel (691214 CE-Marked and US FDA approved) PCR, a real time multiplex PCR was performed according to the manufacturer's instructions (QIAGEN 2020, Germany). In brief, 300 ul of Transport medium liquid samples were loaded manually into the single-use QIAstat-Dx® Respiratory Panel Cartridge. The QIAstat-Dx® Respiratory Panel cartridge and QIAstat-Dx® Analyzer form a closed, fully automated PCR assay; sample preparation, Nucleic acid extraction, amplification and analysis were performed by the QIAstat-Dx® Analyzer 1.0. The QIAstat-Dx Analyzer 1.0 Software interprets the resulting data and process controls and delivers a test report. Total assay run time is 70 minutes. The assay has in-built internal quality control (IC) system and must pass IC for valid result in each run. This Internal Control material verifies all steps of the analysis process, including sample resuspension/homogenization, lysis, nucleic acid purification, reverse transcription and PCR. Automated results are generated by the QIAstat-Dx® Analyzer with Ct value and copy number.

During January 1, 2020 to May 15, 2022, one hundred fifty three routine respiratory panel tests were done. Clinical specimen as nasopharyngeal swabs were collected from outpatients (age ranged from 5 months to 99 years) with flu-like symptoms such as cough, runny or stuffy nose, sore throat, muscle aches, chills and fatigue. Other clinical information such as X-ray results, duration and severity of the illness, clinical diagnosis, or use of antibiotics were not considered.

All samples were de-identified to protect patients' private information except for the age. The presence of SARS-CoV-2 and 21 other respiratory pathogens (influenza A and influenza A subtypes H1N1/2009/pdm09, H1 and H3; influenza B; coronavirus 229E, HKU1, NL63 and OC43; parainfluenza virus 1, 2, 3 and 4; respiratory syncytial virus A/B; human metapneumonovirus A/B; adenovirus; rhinovirus/enterovirus; *Mycoplasma pneumoniae*; *Chlamydophila pneumoniae*, and *Bordatella pertussis*) was determined. This PCR system cannot discriminate rhinovirus/enterovirus. The SARS-CoV-2 targets in the QIAstat-Dx® Respiratory SARS-CoV-2 Panel are ORF1b and the viral membrane envelope (E) gene. Automated results are generated by the QIAstat-Dx® Analyzer with Ct value and copy number.

Results

Among all age groups, the overall positive rate for the URTI pathogens was 67.32% (103/153). The most

frequently detected URTI viruses were human Rhinovirus of Enterovirus family hRV/EnV (16.34%), Flu A (11.12%), Sars-CoV-2 (11.11%), PIVs-(10.45%), RSV (8.50%), other hCoVs (7.19%), hMPV (5.23%) and the other URTI pathogens including, Flu B, Adenovirus, Mycoplasma pneumoniae were also detected but their positive rates were much lower (Figure 1).

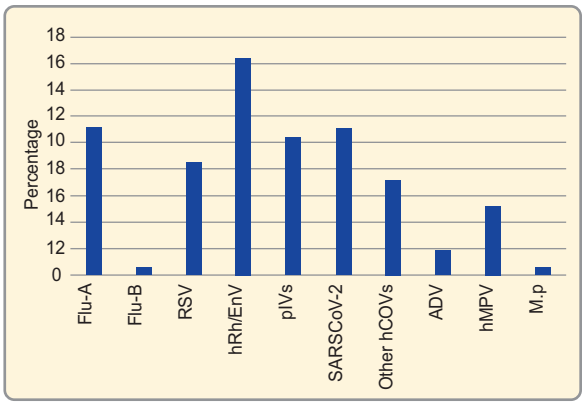

Fig.-1: *Respiratory pathogens detected during January1, 2020—May 15, 2022 FluA Influenza Virus A, FluB Influenza Virus B, RSV Respiratory Syncytial Virus, hRV/EnV human Rhinovirus/Enterovirus, PIVs Parainfluenza Viruses, SARS-COV-2 Severe acute respiratory syndrome corona virus-2, hCoVs human Corona viruses (229E, OC43, NL63, HKU1), ADV Adenovirus, hMPV human Metapneumovirus, M.p Mycoplasma pneumoniae.*

No significant difference was found in the detection rates of overall URTI pathogens among the different age groups (Table 1). Predominant age group are 19-45(77.42%) and 46-65(57.81%). The higher detected pathogen is Rhinovirus of Enterovirus family 25(16.34%) with 3 (42.8%) coinfections among 7 co-infections found in this study period (Table-II and Table-III). Influenza virus was the second 17(11.11%) dominating virus in our cohort. Among 17 influenza A viruses H1N1pdm09 strain was 9(5.88%), H3 was 5 (3.27%) and 3 could not be subtyped. Parainfluenza were detected third (10.45%) dominating virus among the panel. Among parainfluenzas 11 are parainfluenza 1, 4 are parainfluenza 4 and one is parainfluenza 3 (data not shown). Influenza virus was detected in summer period and parainfluenza was in the winter season. These data shows that in COVID-19 pandemic period Rhino/Entero and Influenza is dominating over all other respiratory viruses and among influenza viruses' influenza A pandemic strain (H1N1pdm09) was widely circulating than other strain. *Mycoplasma pneumoniae* was found in only one patient and it was a co-infection with rhino/enterovirus (Table -I and Table-III).

Among all positive cases, the majority (99.03%) were viral infections. About 0.97% were viral and bacterial co-infections. About 4.58% of cases were viral co-infections (Table 2). Co-infections were quite common and found in 6.79% of the positive cases. Among 7 cases of co-infection, two cases were of (RSV+ CoronaNL63) and one case was of SARS-Cov-2 and Parainfluenza virus. Six co-infection cases were 45-65 age group and only one case was 0-5 age group (Table III).

Table-I

Prevalence of RP pathogens among different age groups

Age (Years)	Total Case	Positive	%	Flu. A	Flu. B	RSV	hRh/EnV	PIVs	SARS COV2*	Other HCoVs	ADV	hMPV	M.p	Co-infection
0-5	2	2	100%				50%	50%					50%	Yes -01
6-18	8	5	62.50%	12.50%		12.50%	12.50%					25%		No
19-45	62	48	77.42%	12.90%	1.61%	4.83%	20.96%	9.68%	12.96%	8.06%	1.61%	6.45%		No
46-65	64	37	57.81%	9.38%		12.50%	10.94%	14.06%	8.62%	6.25%	3.13%	3.12%		Yes -06
>65	17	11	64.70%	11.76		5.88%	17.65%		20.00%	11.76%				No
Total (%)	153	103(67.32)		17(11.12)	1(0.65)	13(8.50)	25(16.34)	16(10.45)	15(11.11)	11(7.19)	3(1.96)	8(5.23)	1(0.65)	7(4.58)

*SARS COV2 percentage is from Out of 135 sample.

Table-II

Classification of URTI pathogens among different age groups

Age (Years)	Positive	Viral only (%)	Viral + Viral (%)	Viral+ Bacterial (%)	Bacterial Only (%)	Co-infection (%)
0-5	2	1(50)	0(0.0)	1(50)	0(0.0)	1(50)
6-18	5	5(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
19-45	48	48(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
46-65	37	37(100)	6(16.21)	0(0.0)	0(0.0)	6(16.21)
> 65	11	11(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	103	102(99.03)	6(5.82)	1(0.97)	0(0.0)	7(6.79)

Table-III

Co-infection pathogen and age group distribution

Age group	Number of Co-Infection	Name of Pathogen
0-5 years	1	1. Mycoplasma Pneumoniae Rhinovirus/Enterovirus
46-65 years	6	2. RSV(A+B) Rhinovirus/Enterovirus
		3. RSV(A+B) CoronaNL63
		4. RSV(A+B) CoronaNL63
		5. SARS-COV2 Parainfluenza Virus-1
		6. Adenovirus Human Metapneumovirus A+B
		7. Adenovirus Rhinovirus/Enterovirus

Discussion

Identification of viruses are done either by culture or detecting viral genetic material by PCR. Due to lack of availability of sophisticated cell culture laboratories and the containment levels PCR technology is being used now a days in many countries. Specially, the use of multiplex real-time PCR assays has helped in detecting circulating pathogens and outbreaks and estimating disease burden (9). Though there are few studies about etiologic agents causing acute lower respiratory tract infections in hospitalized patients in Bangladesh (10) this is the first report about pathogen etiologic association in upper respiratory tract in adults and children in Bangladesh. We found hRV/EnV (16.34%), FluA (11.12%), Sars-CoV-2 (11.11%), PIVs-(10.45%), RSV (8.50%), other human corona viruses hCoVs (7.19%),

hMPV (5.23%) are the most common URTI pathogens in patients with flu-like symptoms in our cohort. hRV/EnV is the most prevalent pathogen in our cohort while it is low (6%) in Eastern India (11). Prevalence of Sars-CoV-2 may not actually represent in our cohort as many flu-like symptomatic patients were only screened by COVID-19 RT-PCR test and were not included to be tested by the respiratory panel. Prevalence of respiratory viruses in India are available in different state of India for quite long ago and yearly periodic tracking is going on from 2007. Prevalence in Eastern India of Flu A, Flu B, RSV, PIVs, ADV, hRV, hMPV were found 25%, 22%, 20%, 13%, 8%, 6%, 5% respectively, during 1970 – 2020 (11).

Although case number in the report is not high the majority (99.03%) of the URTIs were of exclusive viral etiology, and only 0.97 % were of bacterial etiology (bacteria + viral co-infections). All co-infections in our cohort are double co-infections, and no cases of triple co-infections were found. Double and triple respiratory co-infections were previously reported in India, China (12). Further study with large sample size is needed to substantiate our findings.

It is interesting to note that only one case of bacterial pathogen association is found out of 153 cases which is a co-infection with hRV/EnV. RSV and hRV/EnV are the two common viral pathogens found in co-infection in our cohort. Thus, these viral etiologic findings added to a large body of evidence supporting the guidelines that recommend against antibiotics prescription to treat URTIs without accurate diagnosis (13). This multiplex assay can confirm the cause of URTIs within short time (assay time <90 minutes) and allows early interventions and thus, may help reduce transmission by interrupting chains.

Limitation of our study is that we could not check the involvement of lower respiratory tract (LRT) and bacterial pathogens associated with LRT. However, pathogen association with pneumonia in under 5 children in Bangladesh is reported mainly viral (77.7%) and bacterial association is found very low (5) and in adult large-scale data is not available.

Conclusion :

This study helped us to understand the diversity of pathogens associated with upper respiratory tract infections in our cohort and warrants further large-scale study in this urban cohort and other geographical areas in the country which may facilitate infection control procedures, consideration of antiviral treatment options and avoiding the inappropriate use of antibiotics.

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