Original Article

ISSN: 1735-0344 *Tanaffos 2022; 21(4): 448-454*

TANAFFOS

Prevalence and Genotypes of Human Rhinoviruses among Iranian Hajj Pilgrims with Severe Acute Respiratory Infection

Somayeh Shatizadeh Malekshahi [1], Fariba Khani [2], Nazanin-Zahra Shafiei-Jandaghi [2], Azadeh Shadab [2], Talat Mokhtari-Azad [2], Jila Yavarian [2, 3]

[1] Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, [2] Virology Department, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, [3] Research Center for Antibiotic Stewardship and Antimicrobial Resistance, Tehran University of Medical Sciences, Tehran, Iran

Received: 24 September 2021
Accepted: 27 June 2022

Correspondence to: Yavarian J
Address: Virology Department, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
Email address: yavarian@tums.ac.ir

Background: Hajj is one of the main challenges of public health and infection control. Hajj-associated respiratory tract infections are very common during the pilgrimage. Studies have shown that human rhinovirus (HRV) is one of the most common causes of respiratory illnesses among pilgrims. The aim of this study was to investigate the prevalence and genotypes of HRV among Iranian pilgrims with severe acute respiratory infection (SARI) during the 2017 Hajj season.

Materials and Methods: Throat swabs or washes were collected from 104 pilgrims with SARI and transported to the National Influenza Center, School of Public Health, Tehran University of Medical Sciences. Specimens were screened for HRV by Nested PCR with primers for 5'UTR, and virus genotypes were determined using PCR with VP4-VP2 primers and sequencing method.

Results: Twenty-one cases were positive for HRV (20.19 %). The HRV species and types of 8 positive samples were: HRV-A21 (1/8, 12.5%), followed by HRV-B91 (3/8, 37.5%) and HRV-C (4/8, 50%) un-typed.

Conclusion: This study showed that HRV has a high prevalence in Iranian Hajj pilgrims. As there is no vaccine or antiviral therapy for HRV, prevention methods are the best way for infection control.

Keywords: Epidemiology; Rhinovirus; Respiratory infection

INTRODUCTION

During Hajj, the "Fifth Pillar of Islam", two to three million Muslims from 184 countries gather in Mecca for religious events. In this annual mass gathering the pilgrims go to different holy places, including Mina, Arafat, and Muzdalifah valleys, where they stay several nights in tent camps (1-3).

The Hajj rituals usually last for one week but the period of pilgrimage lasts up to one month. Spiritual festivals are a potential health risk for the transmission of infectious diseases. As the majority of pilgrims are elderly with chronic underlying diseases, the experience of overcrowded residence, extreme fatigue, and insomnia may greatly increase the risk of acquiring infectious diseases. In this regard, viral respiratory tract infections (RTIs) represent a major concern during Hajj (4, 5).

Human rhinoviruses (HRVs) are traditionally considered as a major causative agent of mild upper respiratory tract illnesses. However, recent studies have shown that some HRVs can infect the lower respiratory tract and cause severe respiratory disease (6). Meanwhile, HRVs could be associated with recurrent wheezing, acute asthma exacerbations, pneumonia, and bronchiolitis (7, 8). Rhinovirus is a member of the *Picornaviridea* family,

genus Entrovirus. The genus is classified into three species, HRV-A, HRV-B, and HRV-C with multiple types within each species (9).

Human Rhinovirus is a non-enveloped, positive-sense single-stranded RNA virus of 7200 bp consisting of a single open reading frame (ORF) with 5' and 3'untranslated regions (UTRs). The ORF encodes a polyprotein that is cleaved by viral proteases to produce 11 proteins including four viral structural proteins of capsid (VP1, VP2, VP3, and VP4) (6, 10).

Human Rhinoviruses are possibly transmitted through infected respiratory secretions, aerosol, and direct contact via hands or indirect contact with fomites (11). Hajj-associated RTIs are very common during the pilgrimage. It has been estimated that more than one-third of Hajj pilgrims suffer from respiratory illness due to respiratory viruses. Studies have shown that HRV is one of the most common causes of respiratory illnesses among pilgrims (12). Of note, there is a paucity of data on the prevalence of HRV among Iranian pilgrims (13).

The aim of this study was to investigate the prevalence and genotype distribution pattern of HRV infection among Iranian pilgrims with severe acute respiratory infection (SARI) during the 2017 Hajj season. To the best of our knowledge, this is the first study of the HRV genotype distribution pattern among Hajj pilgrims.

MATERIALS AND METHODS

Specimen collection

This cross-sectional study was conducted in the National Influenza Center (NIC), School of Public Health, Tehran University of Medical Sciences during the 2017 Hajj season. Throat swabs or washes were collected by the Iranian Ministry of Health from 104 Iranian pilgrims with the diagnosis of SARI and transferred to the NIC along with the patient's information sheets. SARI is defined as an acute respiratory illness manifested by fever (≥38°C), cough, shortness of breath, or difficulty in breathing requiring hospitalization (14). All specimens were stored at -70°C until used for RNA extraction and molecular detection. This study was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1395.876).

RNA extraction

Nucleic acids were extracted from 200 µl of samples using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. The extracted RNA was dissolved in 50 µl of elution buffer and stored at -70 ◦ C for further analysis.

Detection of HRV by nested PCR

HRV was detected by nested PCR using specific outer (RVF1 and RVR1) and inner (RVF2 and RVR2) primers (15) targeting the 5′untranslated region (UTR) of HRV as follows:

RVF1:5'-CACTTCTGTTTCCCCGGAGCGAG-3',
RVR1: 5'-GAAACACGGACACCCAAAGTAGTCGGT-3'
RVF2:5'-CACTTCTGTTTCCCCGGAGCGAGG-3',
RVR2: 5'-CCGCATTCAGGGGCCGGA-3'.

The first round of nested PCR was performed using a One-step RT-PCR kit (Qiagen, Hilden, Germany) yielding a 388 bp amplified product. The second round of PCR was carried out by the Ampliqon kit yielding 283 bp of amplified product according to the manufacturer's protocol.

The first round of PCR reactions was performed in a total volume of 25 µl containing 5 µl RT-PCR Buffer 5X, 1 µl dNTP (2.5 µM each), 1.25 µl of each primer (10 pmol), 1µl one-step enzyme, 7.5 µl RNase free water, and 8 µl extracted genomic RNA. The thermocycling profile consisted of 60 ◦C for 1 min, 50 ◦C for 30 min, and 95 ◦C for 15 min followed by 35 cycles of (94 ◦C for 30 sec, 64 ◦C for 30 sec, and 72 ◦C for 60 ◦C) and 10 min final extension at 72 ◦C. The second round was performed in a total volume of 50 µl containing 25 µl of Taq 2X Master Mix, 2.5 µl of each primer (10pmol), 15 µl RNase-free water, and 5 µl of first-round PCR product. The PCR amplification conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 s, 66°C for 40 s, 72°C for 30 s and a final

extension at 72°C for 5 minutes. PCR products were electrophoresed on 1.5% agarose gel and visualized by DNA-safe stain.

Genotyping of the HRVs based on the VP4-VP2 region

All of the positive samples were tested for the HRV VP4-VP2 region using a One-step RT-PCR kit (Qiagen, Hilden, Germany) using specific forward:

(5'-GGGACCAACTACTTTGGGTGTCCGTGT-3')

and reverse primers:

(5'-GCATCIGGYARYTTCCACCACCAN-3')

yielding a 549- bp product. The PCR reaction was performed in the same way explained in the first round of nested PCR using a One-step RT-PCR kit. The thermocycling conditions consisted of 60 ◦C for 1 min, 50 ◦C for 30 min and 95 ◦C for 15 min followed by 40 amplification cycles (94°C for 1 minute, 58°C for 30 seconds, 72°C for 1 minute) and 5 minutes final extension at 72°C.

Nucleotide sequencing

The PCR products of the HRV VP4-VP2 amplified region were purified according to the manufacturer's instructions using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and then sequenced in the forward and reverse directions using the BigDye Terminator v3.1 Cycle Sequencing Kit in a DNA Sequencer (Applied Biosystems, CA, USA).

Statistical analysis

Data were analyzed using the SPSS software version 22.0. A simple logistic regression analysis was conducted to evaluate the association of independent variables (sex and age) with HRV infection. A P-value less than 0.05 was considered statistically significant.

Genetic characterization and phylogenetic analysis

The nucleotides of the VP4-VP2 region were edited manually to find possible base errors using BioEdit V.7.0.9 software and analyzed using BLAST (Basic Local Alignment Tool) in NCBI.

The nucleotide sequences of the reference HRV strains were retrieved from GenBank and used for multiple alignments and phylogenetic tree construction. The phylogenetic tree was created with the Molecular Evolutionary Genetics Analyses (MEGA10) software using the maximum likelihood method. The topological accuracy and robustness of the tree were estimated by the bootstrap method with 1000 replicates.

RESULTS

Characteristics of the study participants

Half of the 104 Iranian pilgrims enrolled in this study, were male and the other half were female with an age range from 23 to 95 years (median age of 59 years). Approximately 64.4% of the participants were older than 60-year-old that considered to be more vulnerable to the disease.

HRV detection

21 (20.19%) out of the 104 samples collected from pilgrims with SARI were positive for HRVs (Figure 1). The prevalence of HRV was 15.38% in women and 25% in men. There was no statistically significant association between independent variables (gender and age) and HRV prevalence [Table 1 (p=0.328) & Table 2 (p=0.391)].

HRV genotyping

Eight out of 21 HRV-positive samples underwent successful VP4-VP2 gene sequencing (Figure 2). As it was shown in Figure 3, these genotypes were classified as HRV-A21 (1/8, 12.5%), followed by HRV-B91 (3/8, 37.5%) and HRV-C (4/8, 50%) by phylogenetic analysis.

Table 1. Prevalence of human rhinoviruses (HRVs) in Iranian pilgrims with SARI according to gender

	Positive	Negative	P-value
Male	13 (25.00%)	39	
Female	8 (15.38%)	45	0.3287
	21	84	

Table 2. Prevalence of human rhinoviruses (HRVs) in Iranian pilgrims with SARI according to the age groups

	Positive	Negative	P-value
<40	0 (0.00%)	7	
41-50	3 (23.08%)	10	
51-60	3 (21.43%)	11	0.391
61-70	9 (26.47%)	27	
≥ 71	4 (12.12%)	28	
Unknown	2 (66.6%)	1	

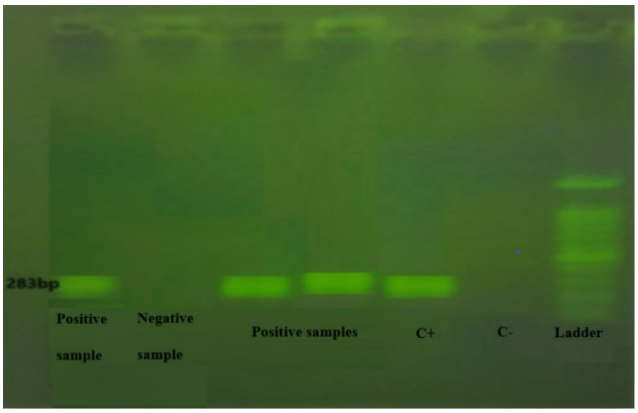

Figure 1. A 283-bp product of the human rhinovirus targeting 5´untranslated region (UTR) was amplified.

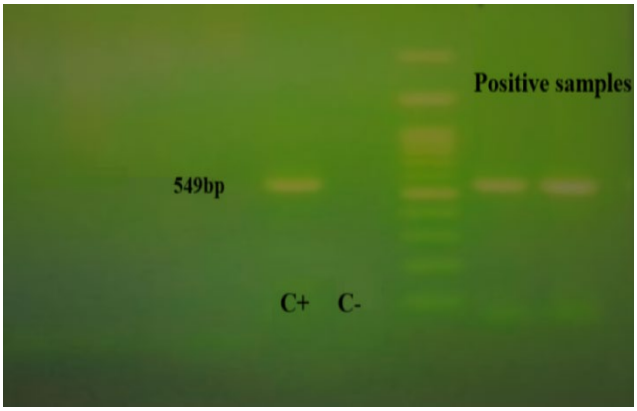

Figure 2. A 549-bp product of the human rhinovirus VP4-VP2 region was amplified.

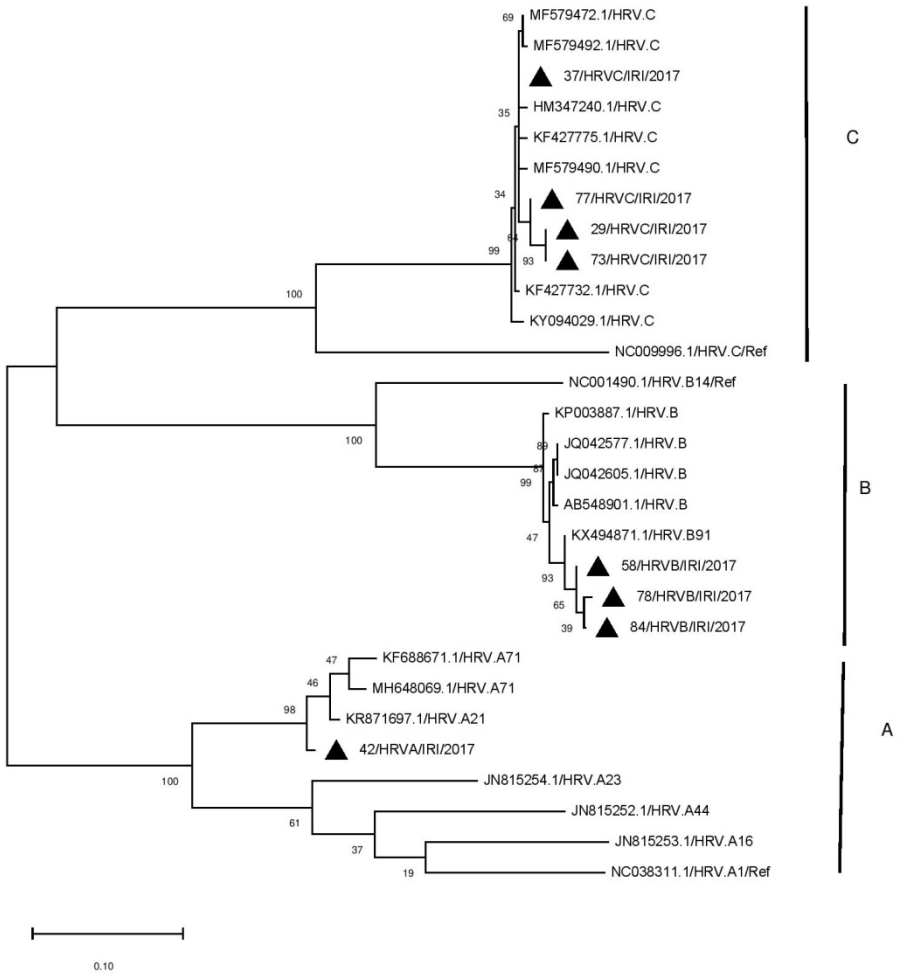

Figure 3. Phylogenetic tree of 8 HRV VP4-VP2 genes from Iran compared with the 17 sequences retrieved from GenBank. The tree was generated by using the maximum likelihood method based on the Tamura-Nei model with 1000 bootstrap analyses. The scale bar indicates the proportion of nucleotide substitutions. The identified HRV-A, B, and C in the Iranian pilgrims are indicated with solid triangles.

DISCUSSION

Respiratory tract infections are a significant health concern during the pilgrimage (16). Mass gathering of Hajj pilgrims increases the risk of acquisition and transmission of various respiratory viruses (17). In this study, we evaluated the prevalence of HRV as well as species distribution among Iranian pilgrims with SARI in 2017.

What is already known is that HRV prevalence ranged from 5.9 to 48.8% among Hajj pilgrims with respiratory symptoms (4). Our result confirms the presence of HRV in 20.19% of throat swab/wash samples with predominant infection in the male population (25% vs.15.38%).

To our knowledge, we are the first to reveal the HRV genotypes among Hajj pilgrims. Therefore, no genotype distribution data are available for HRV species among Hajj pilgrims.

According to previously published studies, the prevalence of HRVs differs among Iranian Hajj pilgrims. In the study by Alborzi et al., HRV was identified in 5.9% of nasal wash samples of 255 pilgrims with symptoms of acute RTIs using nested PCR (13). A relatively high prevalence of HRV was detected by Razavi, et al. in nasopharyngeal secretions of 30% of Iranian pilgrims during 5 consecutive years (18). In one cross-sectional study, nasal and throat swab specimens from 120 hospitalized pilgrims returning from the Hajj presenting with SARI tested for non-influenza respiratory viruses, HRV (15.8%) was the most detected one after adenovirus (20.0%) infection (19). Such disparity can be explained by differences in sample type and seasonal variations. In a randomized controlled trial, HRV was the most common (25%) cause of influenza-like illness among Hajj pilgrims from Saudi Arabia, Australia, and Qatar (3). In a recent study in Saudi Arabia, a total of 126 out of 185 (68.11%) nasopharyngeal swabs of the Hajj pilgrimage tested positive for one or more respiratory viruses during 2019. Human rhinoviruses with 53 cases (42.06%) were the most common detected virus followed by influenza A (H1N1) virus in 27 cases (21.43%) (20). In the study conducted on large groups of pilgrims (451), HRV was the most frequent virus (26.9%) in the pre-and post-Hajj specimens (21). The study by A. Koul et al. pointed out the common prevalence of HRV after influenza and coronaviruses in Indians Hajj and Umrah pilgrims (22).

In order to designate the HRV species, we sequenced and analyzed the VP4-VP2 region. More than half of the positive samples failed for sequencing. It might be a consequence of low viral load in the original sample or due to RNA decay during freezing/thawing cycles. All three HRV species were detected with HRV-C and HRV-B predominance. These results represent the first report on HRV diversity among Hajj pilgrims. The proportions of HRV genotypes in Iranian Hajj pilgrims during 2017 were as follows: 1 HRV-A, 3 HRV-B, and 4 HRV-C. The HRVA genotypes were A-21 and those of HRV-B were B91 and HRV-C was un-typed.

The untypeable HRV-C samples seen in the current study may be due to the presence of new genotypes or possibly, sequencing of one region alone is insufficient for determining the HRV types. The obtained results have some differences from the other studies that may be due to different sampling methods, study types, sample sizes, and types and techniques used.

Our study had some limitations. The study was confined to pilgrims with SARI and cannot be generalized to all Iranian returning Hajj pilgrims while some pilgrims may have infection without symptoms. The study was performed with a limited sample size for a short time. The lack of clinical data was another limitation of this study. Wide population-based studies are required for better clarification of the epidemiology and pathogenesis of HRV species and genotypes.

CONCLUSION

Our study showed that HRV has a high prevalence in Iranian Hajj pilgrims. Since there is no antiviral agent or vaccine for HRV, preventive measures such as personal hygiene, hand washing, use of alcohol-based hand scrubs,

and use of a face mask may reduce the circulation of HRV among pilgrims.

Funding

This study was funded by the School of Public Health, Tehran University of Medical Sciences, grant No. (97-03-27-38331).

Conflict of Interest

The authors have no conflict of interest to declare

Acknowledgment

We would like to thank our colleagues in the Virology Department, School of Public Health, Tehran University of Medical Sciences.

REFERENCES

1. Annan A, Owusu M, Marfo KS, Larbi R, Sarpong FN, Adu-Sarkodie Y, et al. High prevalence of common respiratory viruses and no evidence of Middle East respiratory syndrome coronavirus in Hajj pilgrims returning to Ghana, 2013. ***Trop Med Int Health*** 2015;20(6):807-12.
2. Benkouiten S, Al-Tawfiq JA, Memish ZA, Albarrak A, Gautret P. Clinical respiratory infections and pneumonia during the Hajj pilgrimage: A systematic review. ***Travel Med Infect Dis*** 2019;28:15-26.
3. Barasheed O, Rashid H, Alfelali M, Tashani M, Azeem M, Bokhary H, et al. Viral respiratory infections among Hajj pilgrims in 2013. ***Virol Sin*** 2014;29(6):364-71.
4. Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA. Hajj-associated viral respiratory infections: A systematic review. ***Travel Med Infect Dis*** 2016;14(2):92-109.
5. Benkouiten S, Charrel R, Belhouchat K, Drali T, Salez N, Nougairede A, et al. Circulation of respiratory viruses among pilgrims during the 2012 Hajj pilgrimage. ***Clin Infect Dis*** 2013;57(7):992-1000.
6. To KKW, Yip CCY, Yuen KY. Rhinovirus - From bench to bedside. ***J Formos Med Assoc*** 2017;116(7):496-504.
7. Ozcan C, Toyran M, Civelek E, Erkoçoğlu M, Altaş AB, Albayrak N, et al. Evaluation of respiratory viral pathogens in acute asthma exacerbations during childhood. ***J Asthma*** 2011;48(9):888-93.
8. Midulla F, Pierangeli A, Cangiano G, Bonci E, Salvadei S, Scagnolari C, et al. Rhinovirus bronchiolitis and recurrent wheezing: 1-year follow-up. ***Eur Respir J*** 2012;39(2):396-402.
9. Royston L, Tapparel C. Rhinoviruses and Respiratory Enteroviruses: Not as Simple as ABC. ***Viruses*** 2016;8(1):16.
10. Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. ***Science*** 2009;324(5923):55-9.
11. L'Huillier AG, Tapparel C, Turin L, Boquete-Suter P, Thomas Y, Kaiser L. Survival of rhinoviruses on human fingers. ***Clin Microbiol Infect*** 2015;21(4):381-5.
12. Hashem AM, Al-Subhi TL, Badroon NA, Hassan AM, Bajrai LHM, Banassir TM, et al. MERS-CoV, influenza and other respiratory viruses among symptomatic pilgrims during 2014 Hajj season. ***J Med Virol*** 2019;91(6):911-7.
13. Alborzi A, Aelami MH, Ziyaeyan M, Jamalidoust M, Moeini M, Pourabbas B, et al. Viral etiology of acute respiratory infections among Iranian Hajj pilgrims, 2006. ***J Travel Med*** 2009;16(4):239-42.
14. Fitzner J, Qasmieh S, Mounts AW, Alexander B, Besselaar T, Briand S, et al. Revision of clinical case definitions: influenza-like illness and severe acute respiratory infection. ***Bull World Health Organ*** 2018;96(2):122-8.
15. Lam WY, Yeung AC, Tang JW, Ip M, Chan EW, Hui M, et al. Rapid multiplex nested PCR for detection of respiratory viruses. ***J Clin Microbiol*** 2007;45(11):3631-40.
16. Yavarian J, Shafiei Jandaghi NZ, Naseri M, Hemmati P, Dadras M, Gouya MM, et al. Influenza virus but not MERS coronavirus circulation in Iran, 2013-2016: Comparison between pilgrims and general population. ***Travel Med Infect Dis*** 2018;21:51-5.
17. Al-Tawfiq JA, Gautret P, Benkouiten S, Memish ZA. Mass Gatherings and the Spread of Respiratory Infections. Lessons from the Hajj. ***Ann Am Thorac Soc*** 2016;13(6):759-65.
18. Razavi SM, Mohazzab Torabi S, Salamati P. Treatment and prevention of acute respiratory infections among Iranian hajj

pilgrims: a 5-year follow up study and review of the literature. ***Med J Islam Repub Iran*** 2014;28:31.

19. Sadeghi K, Mokhtari Azad T, Azadeh S, Yavarian J, Shafiei NZ. Detection of non-influenza related viral respiratory infections among returning hajj pilgrims, the Islamic Republic of Iran 2017. ***Oman Medical Journal*** 2020; 35:3.
20. Alsayed SM, Alandijany TA, El-Kafrawy SA, Hassan AM, Bajrai LH, Faizo AA, et al. Pattern of Respiratory Viruses among Pilgrims during 2019 Hajj Season Who Sought Healthcare Due to Severe Respiratory Symptoms. ***Pathogens*** 2021;10(3):315.
21. Hoang VT, Ali-Salem S, Belhouchat K, Meftah M, Sow D, Dao TL, et al. Respiratory tract infections among French Hajj pilgrims from 2014 to 2017. ***Sci Rep*** 2019;9(1):17771.
22. Koul PA, Mir H, Saha S, Chadha MS, Potdar V, Widdowson MA, et al. Respiratory viruses in returning Hajj & Umrah pilgrims with acute respiratory illness in 2014-2015. ***Indian J Med Res*** 2018;148(3):329-33.