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A Preliminary Study on the Frequency of Influenza Infections during the Early 2022 Amid COVID-19 Epidemic in Libya

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Abstract

Background At the time of conducting this study, we were at the peak of the influenza season, and influenza vaccinations were not readily accessible throughout the country. Thus, predisposing many high-risk individuals to influenza infections in a time when coronavirus disease 2019 (COVID-19) was also highly circulating, and the emerging Omicron variant of concern was peaking in many countries worldwide.

Materials and Methods We conducted a brief survey to prospectively estimate the frequency of influenza A and B and severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) in samples received at our laboratories at Libyan Biotechnology Research Center, Tripoli, Libya, between December 1, 2021, and January 31, 2022, for patients complaining of respiratory symptoms using a multiplex reverse transcription-polymerase chain reaction test for SARS-COV-2, influenza A and B, and RSV.

Results We analyzed nasopharyngeal swabs in viral transport media from 2,186 samples. About 27% (589/2186) of study patients tested positive for SARS-COV-2, 2.8% (61/2186) were positive for influenza A virus, 0.18% (4/2186) for influenza B virus, and 1.4% (31/2186) tested positive for RSV.

Conclusions These results revealed that along with COVID-19, influenza infections were also rising. As the COVID-19 pandemic continues, the most significant concern is the development of an influenza outbreak in the upcoming months. Therefore, continuing annual influenza vaccination is critical to increasing population immunity. National influenza surveillance and testing should also be conducted. Furthermore, sequencing and antigenic characterization should be performed regularly. There is a need for continuous monitoring in national laboratories to detect any zoonotic cases and substantial viral evolution.

Keywords
- influenza vaccination
- COVID-19
- surveillance
- influenza activity
- Libya

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Introduction

The World Health Organization (WHO) estimates that one billion influenza cases, three to five million severe cases, and 290,000 to 650,000 influenza-related respiratory deaths occur annually worldwide.[1] Experts in the Centers for Disease Control (CDC), United States, have predicted a severe flu season for the 2021 to 2022 winter because of reduced population-level immunity due to common influenza infections since March 2020 following the coronavirus disease 2019 (COVID-19) pandemic.[2]

Significant declines in influenza activity have been observed worldwide since March 2020, except in certain tropical regions where small activity was identified. Reasons for this were attributed to the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the COVID-19 pandemic, where physical and social distancing, masking, and travel restrictions played an important role.[1]

Between late 2019 and the end of 2020, only 19% of tested respiratory samples in global surveillance were positive for the influenza virus.[3] In North Africa, influenza cases increased in late 2019 in Egypt and Morocco, while influenza detection peaked in January to February 2020 in Algeria and Tunisia. Most infections were influenza A (H1N1) pdm09 viruses representing 69% of all the viruses identified between Nov 2019 and April 2020.[3]

In the northern hemisphere, the influenza season typically starts in October and commonly peaks in January or February. In contrast, in the southern hemisphere, the season starts in their winter months counterparts.[4] Most of Libya lies within the northern hemisphere and a small part within the southern hemisphere, which suggests influenza can be all year round.

In Libya, COVID-19-positive cases reached 38.7% of tested samples during the omicron surge in February 2022. Reverse-transcription polymerase chain reaction (RT-PCR) was the primary diagnostic test employed in public laboratories in Libya, and rapid antigen tests were also used in many point-of-care and filtration units. However, RT-PCR technology was only recently introduced into the majority of these public laboratories during the COVID-19 pandemic, and most other viral illnesses were diagnosed on a clinical basis.

In addition, symptoms of influenza and other respiratory infection are similar to that of COVID-19, making a diagnosis based on clinical presentation alone tricky. Therefore, the causative virus must be tested to confirm the diagnosis.[2]

This preliminary report aims to estimate the frequency of common respiratory pathogens in samples tested at the Libyan Biotechnology Center in Tripoli, Libya, referred for COVID-19 testing for multiple reasons employing a multiplex RT-PCR molecular test.

Materials and Methods

Patient Population

This is a cross-sectional study. We analyzed nasopharyngeal swabs in viral transport media from 2,186 samples that arrived at our laboratories at the Libyan Biotechnology Research Center, Tripoli, Libya, between December 1, 2021, and January 31, 2022. The inclusion criteria were all patients referred for SARS-COV-2 testing with clinical features suggestive of an upper respiratory tract infection and/or pneumonia.

Influenza and SARS-COV-2 Detection

Collected nasopharyngeal swab samples were analyzed by Xpert Xpress Xpress SARS-CoV-2/Flu/RSV (Cepheid, 904 Caribbean Drive Sunnyvale, CA 94089 USA) multiplexed real-time RT-PCR test intended for the simultaneous qualitative detection of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA and the test was run on the GeneXpert Xpress System (Cepheid, United States).

The manufacturer's instructions were followed. Briefly, using the pipette supplied, approximately 300 µL of viral transport media of the swab was added to the assay cartridge. No viral RNA extraction step was needed. The cartridge was loaded into our 16-module GeneXpert Xpress System. The test was completed within approximately 37 minutes for negative samples and depending on viral load for positive ones.

According to the manufacturer, the Xpert Xpress Flu/RSV test was evaluated against multiple strains of influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and RSV subgroups A and B (RSV A and RSV B).[3]

Statistical Analysis

Frequency of cases, estimates of average cycle threshold (Ct), and scatter plot charts were analyzed using the Cepheid GeneXpert system accompanying software.

Results

The majority of tested cases were negative for all viral panels. About 27% (589/2186) of study patients tested positive for SARS-COV-2, 2.8% (61/2186) were positive for influenza A virus, 0.18% (4/2186) influenza B virus, and 1.4% (31/2186) tested positive for RSV (►**Table 1**). Two patients had coinfection of influenza A and SARS-COV-2, and one had coinfection of SARS-COV-2 and RSV. Ten cases were healthcare workers. All patients did not receive influenza vaccination in 2021.

The Ct trend for influenza-positive patients showed that most cases presented during early disease were reflected by lower Ct (mean Ct 23.8 for influenza A and 15.7 for influenza B; ►**Fig. 1**). Ct trend for SARS-COV-2 positive patients showed that most cases were between January 14 and January 29, while RSV cases were scattered throughout the month (►**Fig. 2**).

Discussion

We observed for the first time since the COVID-19 pandemic the emergence of new positive influenza cases in samples

Table 1 Frequency of positive cases for the four viral targets (SARS-COV-2, influenza A and B, and RSV) in the study sample ($n = 2{,}186$) from 01/12/21 to 31/01/22

Target analyte	Frequency of positive cases (%)	Average cycle threshold
SARS-COV-2	589 (26.9)	26.4
Influenza A	61 (2.8)	23.8
Influenza B	4 (0.18)	15.7
RRSV	31 (1.4)	29.5

Abbreviations: RSV, respiratory syncytial virus; SARS-COV-2, severe acute respiratory syndrome coronavirus 2.

from suspected COVID-19 patients in our laboratory using a multiplex assay for SARS-COV-2, influenza, and RSV detection.

We identified 65 cases positive for influenza (~3%). Four cases were influenza B, and all other patients were influenza A. The assay we used only indicates if it is influenza A or B. We estimate a higher proportion because many cases were not tested and probably had a milder illness.

The US most circulating influenza strain is A (H3N1). It would be advantageous to type influenza strains to assess the epidemiological status and track variants. Surveillance performed at the end of 2019 and early 2020 showed that most circulating A(H1N1)pdm09 viruses were the same phylogenetic cluster.[1]

In our sample, few patients were healthcare workers. This subgroup should have received their vaccination for influenza since the beginning of the fall season.

We emphasize the importance of making RT-PCR influenza detection assays more widely available in public and private laboratories. This detection method could be efficiently utilized in most public COVID-19 diagnostic laboratories because they were recently established and equipped with RT-PCR machines and trained personnel. RT-PCR method has a sensitivity of approximately 80% and a specificity of 90%.[4]

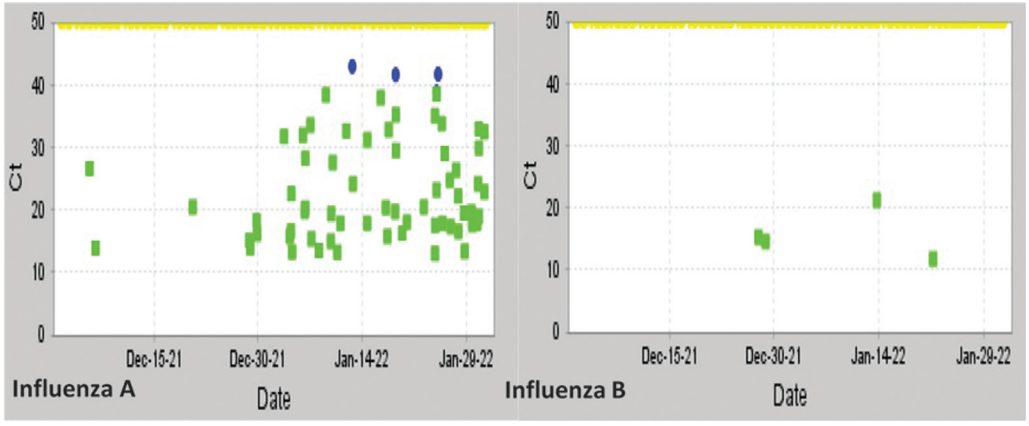

Fig. 1 Cycle threshold (Ct) trend for influenza A and influenza B positive cases. Green squares are positive samples and blue circles are negative samples (above 40 Ct for positivity set by the test manufacturer).

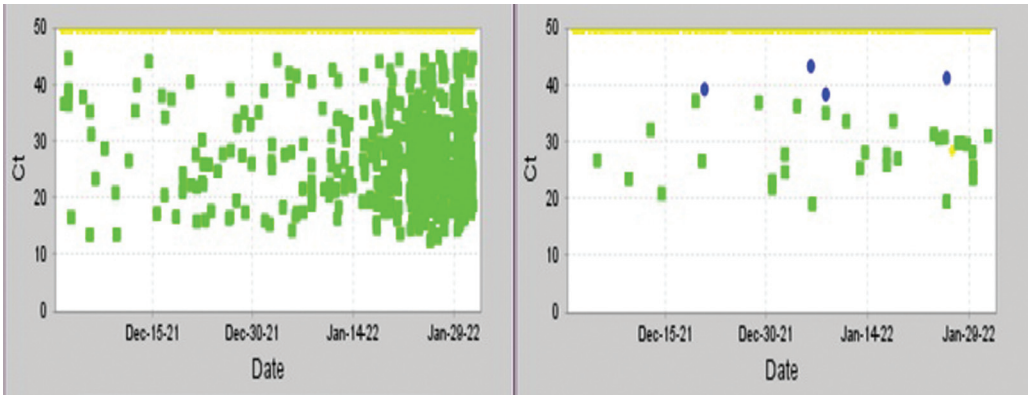

Fig. 2 Cycle threshold (Ct) trend for severe acute respiratory syndrome coronavirus 2 and respiratory syncytial virus positive cases. Green squares are positive samples and blue circles are negative samples (above the 40 Ct for positivity set by the test manufacturer).

There are potential consequences in discovering patients diagnosed with the influenza virus during this time when COVID-19 cases are expected to peak, allowing new highly transmissible Omicron subvariants to emerge. The first issue is the difficulty in making the correct diagnosis without specific viral testing like RT-PCR. Another critical concern is that the same health resources needed for COVID-19 control will also be utilized to fight any influenza activity surge, leading to an extra burden on the health system.

Also, because of the COVID-19 pandemic, all suspected cases of pneumonia are routinely managed as SARS-COV-2 infections risking patients with influenza not receiving the appropriate treatment. The use of corticosteroids in influenza is not recommended because it is associated with higher mortality and nosocomial infections.[5] When influenza patients are admitted to COVID-19 isolation units based on their high-resolution computed tomography findings only without viral testing, there is a risk of coinfection with SARS-COV-2, potentially increasing morbidity and mortality of the patient.

The gold standard for confirming influenza virus infection is RT-PCR or viral culture of samples taken from nasopharyngeal or throat swabs. Rapid diagnostic tests for influenza are available and are becoming more widely used. These tests have high specificity but only moderate sensitivity.[6]

A Libyan national advisory committee on influenza control was established in 2014, and an official recommendation for influenza vaccination was made in 2013.[7] Influenza vaccination in Libya was unavailable in 2021 and the winter of 2022 for the first time since the official recommendation.

The flu season activity most commonly peaks in February and can last into May. Also, during most flu seasons, different flu viruses spread throughout the season. Current US flu vaccines are designed to protect against four different flu viruses: A(H1N1), A(H3N2), and two flu B viruses. The American CDC recommends influenza vaccination despite evidence showing reduced vaccine effectiveness against the current predominant H3N2. However, some protection against those H3N2 viruses was found.[8]

The COVID-19 pandemic hugely reduced the number of influenza detections and adversely affected the generation and reporting of virus characterization because of reduced human and laboratory resources and increased workload due to the response to the pandemic.[9] However, new genetic strains were reported, and some had spread globally.[1,9]

This research, however, is subject to several limitations. Unfortunately, we did not have enough RT-PCR reagents in our laboratory to screen more patients for an extended period and cover hospitals.

As the COVID-19 pandemic continues, the most significant concern is the development of an influenza outbreak in the upcoming months. As mentioned above, population immunity to influenza has decreased because fewer people were exposed to the virus. Therefore, continuing annual influenza vaccination is still critical to increasing population immunity.

In a monthly update by the WHO Eastern Mediterranean Region, the October 2022 statistics of 17 countries that reported influenza data to FluNet and/or EMFLU showed that the number of influenza cases increased in October compared with the previous 4 months.[10]

Due to the circulation of both influenza and SARS-COV-2 viruses during this season, coinfection is possible and should be considered. Also, it is crucial to develop updated guidelines for managing patients with suspected viral pneumonia during this time and offer education programs for healthcare providers to ensure proper diagnosis and treatment of such cases.

Conclusions

The results of the present study revealed that influenza infections were also rising along with COVID-19. As the COVID-19 pandemic continues, the most significant concern is the development of an influenza outbreak in the upcoming months. Therefore, continuing annual influenza vaccination is critical to increasing population immunity. There is a need for continuous monitoring in national laboratories to detect any zoonotic events and important viral evolution. National influenza surveillance and testing should be conducted. Furthermore, sequencing and antigenic characterization should be performed regularly. Providing molecular testing for respiratory viruses including influenza and respiratory syncytial virus in addition to SARS-COV-2 could not be more emphasized to prevent a potential tripledemic.

Authors' Contributions

Inas M Alhudiri and Zakarya Abusrewil conceptualized and designed the study. Saad R Saad, Asel Omar Amer, Mohamed Nasir Bin Abdallah, Mohamed Mustafa Elghazal, and Mohamed Hamed Said helped in data collection. Fawzi O. Ebrahim, Mohamed S Abusanina, Mohammed Ben Elfghi, and Mohamed Musbah Abdusalam were involved in laboratory analysis. Salah Edin El Meshri, Fawzi O. Ebrahim, and Inas M Alhudiri contributed to data analysis and interpretation. Inas M Alhudiri and Zakarya Abusrewil wrote the manuscript. Adam Elzagheid edited and revised the manuscript.

Compliance with Ethical Principles

All procedures performed in studies involving human participants followed the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Libyan Biotechnology Research Center Bioethics Committee (Ref No. BEC-BTRC 8–2020).

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No funding was received for this study.

Conflict of Interest

None declared.

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