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Original Article

Co-infections of SARS-CoV-2 with respiratory syncytial virus and human influenza A in patients with symptoms of COVID-19 in Ghana: A retrospective study

Kwabena Obeng Duedu [a,b,*], Jones Gyamfi [c,d], Reuben Ayivor-Djanie [a,e], Godknows Afenya [a,1], Isaac Buertey Agbuglah [a,1], Hubert Kwame Agbogli [a], Priscilla Essandoh [a], Seraphine Kugbemanya [a], Theophilus Koku Adiku [a]

[a] Department of Biomedical Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, PMB 31, Ho, VH0194, Ghana
[b] College of Life Sciences, Birmingham City University, City South Campus, Birmingham, United Kingdom
[c] Department of Medical Laboratory Sciences, School of Allied Health Sciences, University of Health and Allied Sciences, PMB 31, Ho, VH0194, Ghana
[d] School of Health & Life Sciences, Teesside University, Middlesbrough, United Kingdom
[e] West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Legon, Ghana

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ABSTRACT

Background: During the COVID-19 pandemic the aetiology of respiratory illnesses were narrowed to SARS-CoV-2. This prevented diagnosis of other pathogens and patients were not notified of the accurate diagnosis of their illnesses when SARS-CoV-2 was absent. It is therefore important to look back and determine what else was present but was missed.
Objective: This retrospective study sought to gain insights into prevalence of respiratory syncytial virus (RSV) and influenza A alongside SARS-CoV-2 in patients who reported with clinical symptoms of respiratory illnesses.
Methods: Samples from patients who had reported of respiratory symptoms were selected at random from a pool. RNA was extracted and RT-PCR was performed for SARS-CoV-2, RSV and Influenza A in parallel. Data on the clinical symptoms was extracted from case-base forms and analysed.
Results: Of the 400 symptomatic samples tested, prevalence of SARS-CoV-2, influenza A and RSV was 20.3 %, 2.0 % and 0.5 % respectively. Only one sample tested positive for SARS-CoV-2 and influenza A. About 77 % of the symptomatic cases did not test positive for any of the three agents. Cough (79 %) was the most common symptom followed by fever and chills, headache, sore throat and runny nose.
Conclusion: The large proportion of symptomatic cases that tested negative for all three respiratory viruses raises a flag and a need for more investigations into the actual burden of respiratory aetiologic agents during the pandemic. With the low levels of co-infections, parallel testing may not be needed however, a strong case for multiplex tests for respiratory agents exists.

1. Introduction

The novel coronavirus disease 2019 (COVID-19) pandemic was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) on January 30, 2020 [1]. Although a respiratory disease, COVID-19 has been reported to affect more than the respiratory system making it a multisystem disease [2,3]. A wide spectrum of symptoms has since been described to be associated with the infection. According to WHO the most common symptoms of COVID-19 are fever, dry cough, and fatigue whereas other less common symptoms include sore throat, diarrhoea, headache, aches and pains, nasal congestions, red-eye, or a skin rash [4].

Co-infections with COVID-19 have been reported in various studies. In a systematic review and meta-analysis of such studies, the pooled prevalence of bacterial co-infections and for viral co-infections was reported as 20.97 % (95 % CI: 15.95–26.46) and 12.58 % (95 % CI:

* Corresponding author. College of Life Sciences, Birmingham City University, City South Campus, Birmingham, United Kingdom.
E-mail addresses: kduedu@uhas.edu.gh, kwabena.duedu@bcu.ac.uk (K.O. Duedu).
[1] These authors contributed equally.

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7.31–18.96) respectively [5]. Another systematic review and meta-analysis reported that influenza type A (InfV A), rhinovirus and non-SARS-CoV-2 coronaviruses were the frequent viruses among co-infected patients [6]. Other studies reported viral co-infection rates of 11.6 % and 12.5 % among COVID-19 patients [7,8]. RSV has also been reported in other studies as an important prevalent pathogen in COVID-19 patients [9–11]. Coinfections can lengthen hospital stays, increase the risk of developing acute respiratory distress syndrome, and necessitate a greater level of care [12,13].

Due to the fear and stigma associated with the pandemic, persons with respiratory symptoms are first regarded COVID-19 suspects. Delays in testing and getting confirmation [14,15] most likely will lead to exacerbation of the clinical condition and possibly death. Public health efforts should therefore focus on establishing the epidemiology other respiratory pathogens among COVID-19 suspected patients to guide clinical management. This study aimed at determining prevalence of InfV A and RSV among suspected COVID-19 patients in Ghana.

2. Methods

2.1. Ethical considerations

The study was approved by a Research Ethics Committee (details removed due to double blind peer review requirements). Written informed consent was waived as the study did not interact with study participants and rather worked on anonymised stored samples and data. All methods were carried out in accordance with relevant guidelines and regulations as approved by the ethics committee.

2.2. Population and samples

A retrospective cross-sectional study was conducted on 400 respiratory specimens submitted for COVID-19 testing between 24th February and May 31, 2021. A total of 5411 samples were tested of which 2794 had symptoms of respiratory illnesses indicated on the accompanying COVID-19 Case Base Forms. Since RNA was to be re-extracted, left over samples whose quantity was not adequate for extraction were omitted. Hence, we selected the first 400 which had the most adequate volume of sample for extraction.

2.3. RNA extraction and polymerase chain reactions

The QIAamp Viral RNA Mini kit (QIAGEN N.V., Netherlands) was used to extract nucleic acid from sputum and nasopharyngeal swab samples according to the manufacturer's instructions and eluded in 50 µl. Detection of SARS-CoV-2 viral RNA was performed using the Da-An-Gene 2019 Novel Coronavirus (2019-nCoV) real time PCR (RT-PCR) detection kit (Da An Gene Co. Ltd. of Sun Yet-Sen University, China) according to the manufacturer's instructions. Positive samples for Influenza A and RSV were obtained from the Virology Department of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. These two pathogens were selected based on availability of primers, positive controls and resources at the time of the study.

For RSV and Influenza A testing, the SuperScript™ III One-Step RT-PCR System (Invitrogen, North America) was used according to the manufacturer's instructions. RSV detection was done using primers AGB490 (5′-ATGATTWYCAYTTTGAAGTGTTC-3′) and F164 (5′-GTTATGACACTGGTATACCAA CC-3′) reported previously [16]. and M30F2/08-5′-ATGAGYCTTYTAACCGAGGTCGAAACG-3′ and M264R3/08-5′-TGGACAAANCGTCTACGCTGCAG-3′ for Influenza A [17]. The cDNA synthesis was done in one cycle at 50 °C for 20 min and 94 °C for 2 min. This was followed by a 40-cycle reaction at 94 °C for 1 min, 55 °C for 30 s, and 68 °C for 42 s for RSV and 94 °C for 1 min, 55 °C for 30 s, and 68 °C for 30 s. A final extension of 68 °C for 6 min was performed after the 40 cycles. Final extension for both reactions was performed at 68 °C for 6 min. All reactions were performed on the ABI 7900HT Real-Time PCR System (Applied Biosystems, New Jersey, USA). The PCR products were visualized on 2 % agarose gel stained with ethidium bromide and visualized using the UVITEC Gel Documentation System (Uvitec Ltd, UK). The product sizes were 244bp and 688bp for Influenza A and RSV respectively.

2.4. Statistical analysis

Demographic and clinical data on individuals, along with results from the RT-PCR were entered in an Excel spreadsheet (Microsoft 360). Confidence intervals of proportions were calculated using GraphPad Prism based on the modified Wald method.

3. Results

Of the 400 samples tested, 54 % were from females and 45.8 % from males. The prevalence of SARS-CoV-2 was 20.3 % (95 % CI 16.59–24.47 %). The prevalence of influenza A was 2.0 % (95 % CI 0.95–3.97 %) and RSV 0.5 % (95 % CI 0.01–1.93 %). Only one individual tested positive for both SARS-COV-2 and Influenza A. The most common symptom in the group was cough (79.0 %, 95 % CI 74.73–82.72 %) followed by fever (49.5 %, 95 % CI 44.63–54.38 %), headache (49.25 %, 95 % CI 44.38–54.13 %), sore throat (31.8 %, 95 % CI 27.38–36.47 %) and runny nose (25.8 %, 95 % CI 21.7- - 30.26 %). In all, 2.8 % (95 % CI 1.48–4.92 %) of the group reported with all five respiratory symptoms whereas 4.3 % (95 % CI 2.62–6.75 %), 5.5 % (95 % CI 3.62–8.23), and 14.8 % (95 % CI 11.59–18.58 %) reported with four, three and two symptoms respectively. None of the samples which came from patients who reported all symptoms was positive for any of the three pathogens. The proportion of symptoms among the SARS-CoV-2 positive and negative cohorts as well as between genders have been provided in Fig. 1. Details of symptoms reported and infection with the three respiratory viruses have been provided in the supplementary file. There were 77 % (95 % CI 72.62–80.86 %) of the suspected cases who had at least one symptom of respiratory illness but did not have SARS-CoV-2, Influenza A or RSV.

4. Discussion

The panic with the COVID-19 pandemic globally has died down although cases and new variants continue to arise. With life back to 'normal', it is time to take stock of what happened and learn from the mistakes as well as the successes. During the pandemic, all attention was directed to SARS-CoV-2 as the most probable aetiology of respiratory illnesses in Ghana and many other places [18,19]. For example, the World Health Organization Global Tuberculosis Report estimated that about 4.2 million people who developed TB in 2021 were not diagnosed or notified [20]. It was as though no other aetiologic agent could be responsible for the symptoms patients faced. At the COVID-19 Testing Centre, we observed that a good number of patients with clinical symptoms of respiratory illnesses did not test positive for SARS-CoV-2. The impact of this was that those patients did not receive treatment because, health personnel were somehow 'afraid'. This was particularly so because of the limited number of personal protective equipment that were available to personnel to handle patients.

In this study, we noticed a large proportion of patients who presented with at least one clinical symptom did not have any of the three respiratory viruses as the aetiology. We recorded a much lower prevalence of respiratory viral co-infection with SARS-CoV-2 compared to the 8.4 % recorded in a study from the UK [21]. A Chinese study also reported 45.5 % co-infection of SARS-CoV-2 with influenza IgM testing [22]. An Iranian study also reported almost 25 % of influenza with SARS-CoV-2 alongside RSV, parainfluenza viruses, adenoviruses, among others [23]. In a systematic review of 21 studies that looked at SARS-CoV-2 and influenza co-infections, the mean prevalence of co-infections was 16.3 % (0.04 %–58 %) [24] which well covers the prevalence seen in this Ghanaian study. There are very few reports of respiratory pathogens

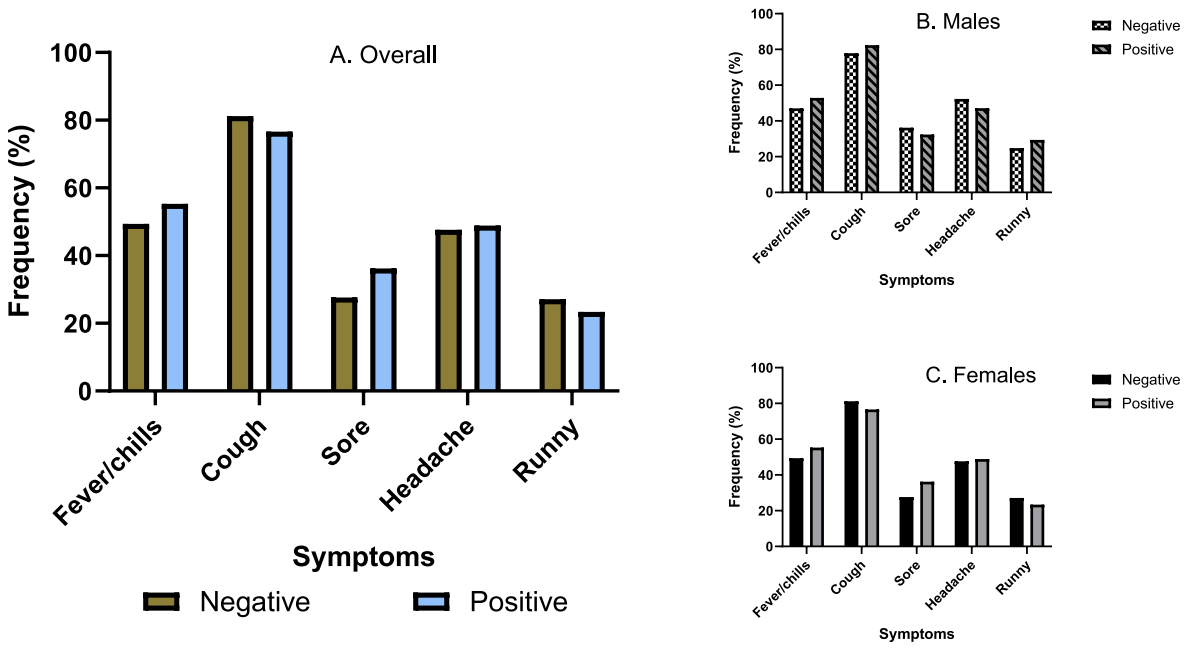

Fig. 1. Frequency of symptoms among study cases. A: Symptoms among SARS-CoV-2 positive and negative cases; B and C: Symptoms groups by gender. There was no significant difference between the proportions of symptoms among the SARS-CoV-2 positive and negative cohorts as well as between genders.

from both adults and children in Ghana. Most studies have focused on children due to the high level of morbidity and mortality in children as a result of respiratory infections [25–27]. Some of the reported circulating respiratory viruses from these studies include RSV, human metapneumovirus (HMPV), rhinoviruses, enteroviruses, parainfluenza and influenza. RSV is one of the most frequently encountered.

Our study is one of the few to investigate co-infections in Ghana and Africa in general. Although these are simple studies, they are very important for public health management and decision making. So far, the data does not suggest that simultaneous testing of SARS-CoV-2 and other respiratory viruses was needed. However, the large group of cases with clinical symptoms and yet negative for SARS-CoV-2 and other respiratory viruses investigated suggest that more investigations are needed targeting other respiratory pathogens to establish respiratory epidemiology during the pandemic. A need for diagnostics for respiratory panels is thus high. Routine surveillance of respiratory pathogens is not a public health programme, but the SARS-CoV-2 pandemic has taught us that, we should probably be looking into doing that. Through the various programmes instituted for the COVID-19 pandemic one can say that significant capacity building now exists in most countries to detect and manage respiratory infections. It is therefore natural to consider using this opportunity to consider making respiratory pathogen surveillance a routine public health programme. Reports of shedding of respiratory pathogens like SARS-COV-2 in faeces and wastewater [28–30] further suggest that, those samples could aid in environmental monitoring.

5. Limitations

This study reports retrospective data on co-infections among stored samples collected during the COVID-19 pandemic. This is however important because, we still do not understand fully the events during the pandemic and at the time, attention was on investigating SARS-COV-2 and not other potential pathogens. Another limitation to this study is that the sample size is not large. Ideally a large sample size will be epidemiologically necessary. Due to limited resources (as this study was not funded), we had to limit the numbers. Considering that we are still learning post-pandemic what went well or wrong in order to be better positioned for future pandemics, the data despite the small sample size is relevant. Furthermore, data from sub-Saharan Africa and Ghana in particular on co-infections is missing in the bigger picture and hence, this study is important.

6. Conclusion

In conclusion, we found that more than a third of patients who presented with respiratory symptoms did not have SARS-CoV-2, influenza or RSV infections in our cohort. his study has revealed that a large proportion of patients suspected. Co-infection with influenza A was found on only one of the samples in the cohort and none was recorded for RSV. The findings suggest that, there could be more pathogens that were circulating alongside SARS-CoV-2. It underscores the need to go back where possible and where resources are available to get a sense of the epidemiology of respiratory pathogens during the pandemic. With the low levels of co-infections, a case for simultaneous testing is unlikely however, where multiplex tests are available, it will be cheaper to test for multiple pathogens whilst aiding health care provided to provide more tailored and relevant management to patients.

Consent for publication

Not applicable.

Availability of data and materials

All study data collected in this study are presented in this publication.

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Ethical considerations

The study was approved by the Research Ethics Committee of the University of Health and Allied Sciences, Ho with protocol reference numbers UHAS-REC A.12 [16] 20–21 and UHAS-REC A.12 [9] 20–21.

Written informed consent was waived as the study did not interact with study participants and rather worked on anonymised stored samples and data. All methods were carried out in accordance with relevant guidelines and regulations as approved by the ethics committee.

Population and samples

We conducted the study at the University of Health and Allied Sciences COVID-19 Centre. The Centre is responsible for PCR testing of samples from the Volta and Oti Regions of Ghana and parts of the Eastern Region. A retrospective cross-sectional study was conducted on 400 respiratory specimens that had been submitted to the Centre for COVID-19 testing. The samples were selected randomly from those for which symptoms of respiratory illnesses had been indicated on the accompanying Ghana Health Service COVID-19 Case Base Forms.

CRediT authorship contribution statement

Kwabena Obeng Duedu: Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Jones Gyamfi:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **Reuben Ayivor-Djanie:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **Godknows Afenya:** Methodology, Investigation, Data curation. **Isaac Buertey Agbuglah:** Methodology, Investigation, Data curation. **Hubert Kwame Agbogli:** Methodology, Investigation. **Priscilla Essandoh:** Methodology, Investigation. **Seraphine Kugbemanya:** Methodology, Investigation. **Theophilus Koku Adiku:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2024.101463.

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