



Detection of SARS-CoV-2 among travellers entering Ghana through the Kotoka International Airport at the peak of the COVID-19 pandemic: 2021 to 2022

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Abstract

Background: The emergence of COVID-19 disrupted health systems and programs, and the impact of its spread and consequences led to the shutdown of country borders worldwide. As part of the Ghanaian government's measures to hasten the opening of its borders, a surveillance system was instituted at the Kotoka International Airport (KIA) to detect and monitor SARS-CoV-2 variants imported into the country.

Objective: This study aimed to screen international travellers arriving in Ghana for SARS-CoV-2, characterise imported viral variants, and assess the role of rapid antigen testing and mandatory isolation in preventing community transmission.

Methods: All arriving passengers at the KIA, except for children under five (5), were required to undergo mandatory antigen testing. The data presented in this study were from 2021 to 2022. Reactive travellers to SARS-CoV-2 antigen testing were placed under compulsory isolation, and retesting was conducted using real-time polymerase chain reaction (RT-PCR). The detected viruses were further sequenced using Oxford Nanopore technologies to identify variants.

Results: Of the travellers arriving at the KIA, 3331 were SARS-CoV-2 Ag+, and 73.73% (n = 2456/3331) were confirmed positive by RT-PCR. The mean age was 37 years, with most cases detected between the ages of 21 and 50. Travellers from Nigeria, Italy, and France had the highest reactive cases upon arrival. All the WHO-labelled variants, including the recombinant variant, were detected upon characterisation. Vaccination among reactive travellers was approximately 32% (n = 1065/3331).

Conclusion: This study highlights the importance of establishing health surveillance systems at points of entry: these effectively monitor disease importation and transmission. Also, this study reiterates the vital role rapid antigen testing plays in the event of a public health crisis.

Keywords: COVID-19 pandemic, Ghana, antigen testing, PCR

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INTRODUCTION

In March 2020, Ghana's National Influenza Centre (NIC) detected the first two imported SARS-CoV-2

cases in the country [1]. These cases had epidemiological links to countries that had already recorded SARS-CoV-2 infections. Ghana subsequently recorded six confirmed imported cases, with no local transmission detected as of March 15, 2020 [2]. The Ghana government issued an order on March 12, 2020, banning travellers who had recently visited countries with 200 or more documented COVID-19

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cases from entering the country [1,3] in response to a global campaign to reduce the importation of COVID-19 cases into less affected nations [2]. The directive took effect on March 17, 2020, affecting 24 countries. The subsequent increase in the number of imported cases led to the complete closure of land, sea, and air borders on March 22, 2022, to prevent importation and limit the spread of the virus. However, on September 1, 2020, Ghana reopened its main air border, the Kotoka International Airport (KIA), with land and sea borders still closed. Travellers arriving at the KIA were required to present a negative COVID-19 reverse transcription polymerase chain reaction (rt-PCR) test result from an accredited laboratory in their country of origin obtained within 72 hours of departure. Also, arriving passengers, except children under 5 years of age, were required to undergo a mandatory COVID-19 rapid antigen test (RAT). Those who tested positive for COVID-19 received further clinical assessment and treatment under mandatory isolation [4–6]. Persons who were isolated were released after a negative RT-PCR. These strategies were to help prevent further importation of SARS-CoV-2 by travellers into the country.

As of August 31, 2020, Ghana had recorded 44,015 laboratory-confirmed cases of COVID-19, with a case fatality rate of 0.63% ($n = 276/44,015$) [4]. From January 2021 to March 2022, the government of Ghana (GoG), in collaboration with the Ghana Health Service (GHS)/Ministry of Health (MOH) and the NIC at the Noguchi Memorial Institute for Medical Research (NMIMR), monitored importation of SARS-CoV-2 variants into the country through the KIA. The objective of this study was to screen and detect SARS-CoV-2 at Ghana's primary air border point, isolate positive cases to prevent community transmission, and identify high-risk

variants circulating globally. Conceptually, this study was carried out in the public health framework of point-of-entry surveillance, which posits that early detection and containment of imported cases are critical to reducing community transmission and informing national response strategies. Border surveillance has been demonstrated in previous epidemics (such as SARS in 2003 and Ebola in 2014) to be an essential layer of defence against the rapid dissemination of pathogens across countries [7,8]. By applying this framework, we sought to generate evidence on the utility of rapid antigen testing and mandatory isolation as effective pandemic interventions. This study reports on SARS-CoV-2 variants detected among travellers in mandatory isolation from January 2021 to March 2022.

MATERIALS AND METHODS

Mandatory testing for SARS-CoV-2 among travellers arriving at KIA began in September 2020 and continued through March 2022. From January 2021, the NIC, in collaboration with the Ghana Health Service and KIA, tested and sequenced respiratory samples from travellers who tested Ag+ (antigen-positive) and were in mandatory isolation. Nasopharyngeal swabs were collected and subjected to SARS-CoV-2 RAT using an automated immunoassay analyser (AFIAS-6) with its accompanying cartridges (AFIAS cartridges) (Boditech Med., Chuncheon-si, Gangwon-do, Republic of Korea). Travellers testing Ag+ were placed in isolation at selected facilities. Subsequently, oro- and nasopharyngeal swabs were collected on the third (3rd) day of isolation and were required to rt-PCR testing [1]. All rt-PCR tests were carried out at the NIC-NMIMR, and the results were communicated to the attending physicians at the isolation facilities. rt-PCR-positive samples with cycle threshold

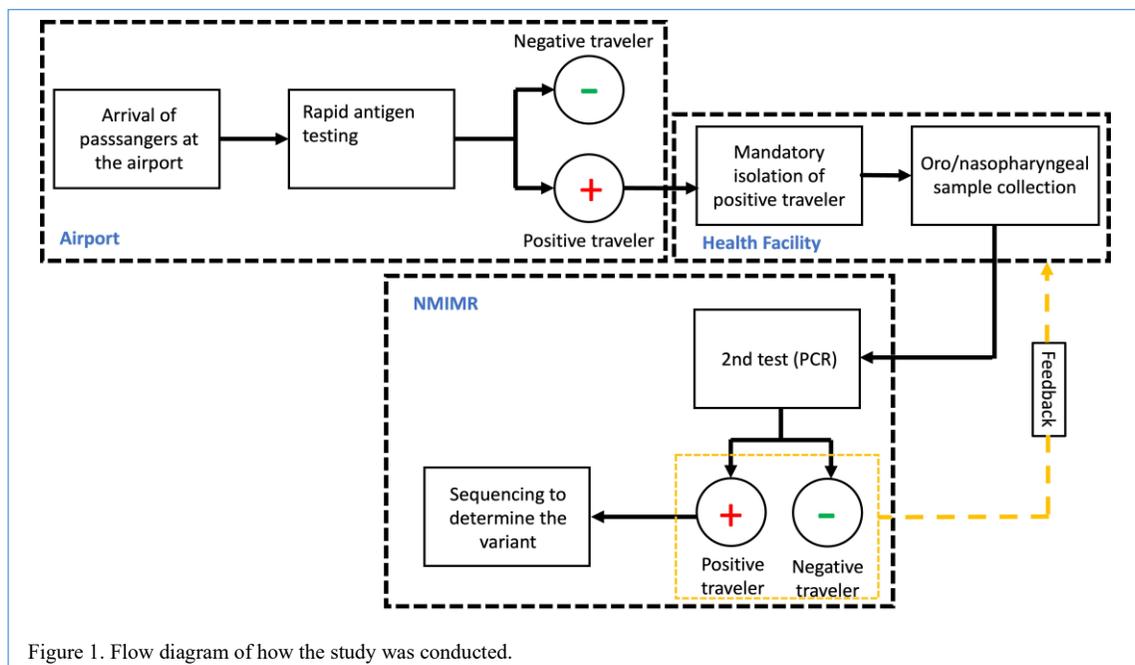


Figure 1. Flow diagram of how the study was conducted.

(Ct) values below 30 were sequenced using Oxford Nanopore technologies to determine SARS-CoV-2 variants that may have been imported (Figure 1). Demographic information, country of origin, vaccination status among travellers, age, and sex were recorded.

Sample processing

Collected oropharyngeal and nasopharyngeal swabs were transported to the NIC using the triple-packaging system to maintain the cold chain. Upon receipt, laboratory identifiers were assigned. Following the manufacturer's instructions, ribonucleic acid (RNA) was isolated from the samples using the QIAamp viral RNA mini kit (Qiagen, Germany). Reverse transcription real-time PCR was then performed using commercially available kits (ModularDx (TIB MOLBIOL, Germany) and VeriQ PCR 316 nCoV-QS (MiCo Biomed, Korea) with specific primers and probes targeting SARS-CoV-2 nucleocapsid (N), the open-reading frame lab (ORF lab), and the envelope (E) genes, following the manufacturer's instructions, and the results were then interpreted. A sample was considered positive when the cycle threshold value was < 40.

Sequencing

The Artic amplicon (Integrated DNA Technologies Inc., USA) sequencing protocol and Oxford Nanopore NTechnology (ONT) MinION (Oxford Nanopore Technologies, UK) were used for the SARS-CoV-2 sequencing. The QIAamp viral RNA isolation kit was modified to use 280 μ L of starting material, followed by elution in 35 μ L of elution buffer. Complementary DNA (cDNA) was synthesised from isolated RNA using Lunascript RT (New England Biolabs Inc., USA). cDNA was subjected to a multiplex PCR using the Artic nCoV-2019/v3 primers [9]. PCR products were visualised by gel electrophoresis for the presence of 400 bp fragments.

Products were quantified using the QubitTM dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). Amplicons were subjected to end repair with the Ultra II End Prep (New England Biolabs Inc., USA). DNA fragments were barcoded using the NBXX barcode kit (ONT, UK). The barcoded library was purified with Agencourt AMPure XP beads (Beckman Coulter Inc., USA). Adapters were then added using Adapter Mix (AMII) (ONT, UK) with Quick T4 DNA ligase (New England Biolabs Inc., USA). A second cleanup was prepared using Agencourt AmPure XP beads (Beckman Coulter Inc., TX, USA). DNA libraries were subsequently loaded onto a MinION.

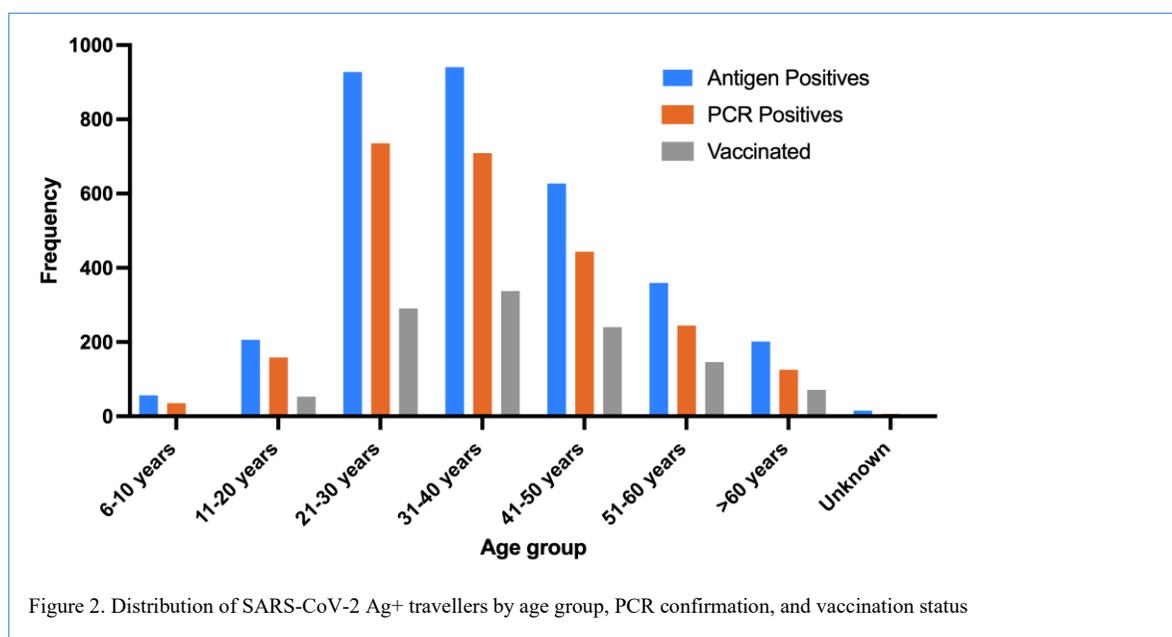
Data analysis

Data were processed and analysed using Microsoft Excel 16, STATA 16, and QGIS 3.28. The Arctic field bioinformatics pipeline assembled the nanopore sequencing data into consensus genomes. Reads obtained were mapped to the reference genome MN908947.3. Mapped genomes were converted to Binary Alignment Map (BAM), sorted, and indexed using samtools (version 1.10). Nanopolish version 0.13.2 was used for variant calling.

RESULTS

Demographics of travellers

From January 2021 to March 2022, a total of 3,331 passengers captured by this study were reactive to SARS-CoV-2 RAT out of 885,426 tested at KIA. The mean age of SARS-CoV-2 Ag+ travellers was 37 ± 13 years. Age groups 21 - 30 years (27.32%, $n = 910/3331$) and 31 - 40 years (27.59%, $n = 919/3331$) accounted for the largest proportions (27%) compared to the other age groups. Males



accounted for approximately 60.43% ($n = 2013/3331$) of the Ag+ passengers, females for approximately 39.33% ($n = 1,310/3331$), and 8 with no sex stated. Of the travellers testing Ag+ during this period, 34.22% ($n = 1140/3331$) had received at least one dose of a vaccine upon arrival. Of the 3,331 antigen-reactive travellers, 73.73% ($n = 2456/3331$) were confirmed SARS-CoV-2 positive using rt-PCR after three days of quarantine. PCR positive rate was recorded in those aged 60 years and above, and the highest in those aged 21 - 30 years, followed by 31 - 40 years (Figure 2). No fatal cases were recorded.

Countries of origin of arriving travellers to KIA

From January 2021 to March 2022, travellers testing SARS CoV-2 Ag+ at the KIA, Accra, arrived from 115 countries. Most detected cases were from travellers coming from Nigeria (22.87%, $n = 762/3331$). The rest included the United Kingdom (UK) (7.02%, $n = 334/3331$), the United States of America (USA) (9.85%, $n = 328/3331$), the United Arab Emirates (UAE) (7.02%, $n = 234/3331$), South Africa (4.62%, $n = 154/3331$) and Cotê d'Ivoire (4.53%, $n = 151/3331$). The majority of Ag+ travellers had a history of travel from African countries (51.18%, $n = 1705/3331$),

followed by Europe (20.26%, $n = 675/3331$), and the least from South America (0.54%, $n = 18/3331$) and Australia (one case). More than one variant was detected from travellers from Africa, Asia, Australia, Europe, and North America, while only one variant was detected from South America (omicron) and Australia (B.1.177) (Figure 3).

Imported variants of SARS CoV-2 and community transmission

Ghana experienced four waves of COVID-19, each characterised by a distinct strain of SARS-CoV-2. The initial waves, spanning from April to December 2020, were primarily influenced by early lineages of SARS-CoV-2 (B.11 and B.1.1). The second wave, occurring from January to April 2021, was caused by the Alpha variant (B.1.1.7) and was detected among travellers arriving in the country before community spread (Figure 4). From May to November 2021, Ghana encountered the third wave, marked by infections caused by the delta variant (B.1.617.2) and its sub-lineages. The last epidemic wave occurred between November and December 2021, with the Omicron variant (B.1.1.529) being the dominant strain causing COVID-19 infections in the country [10].

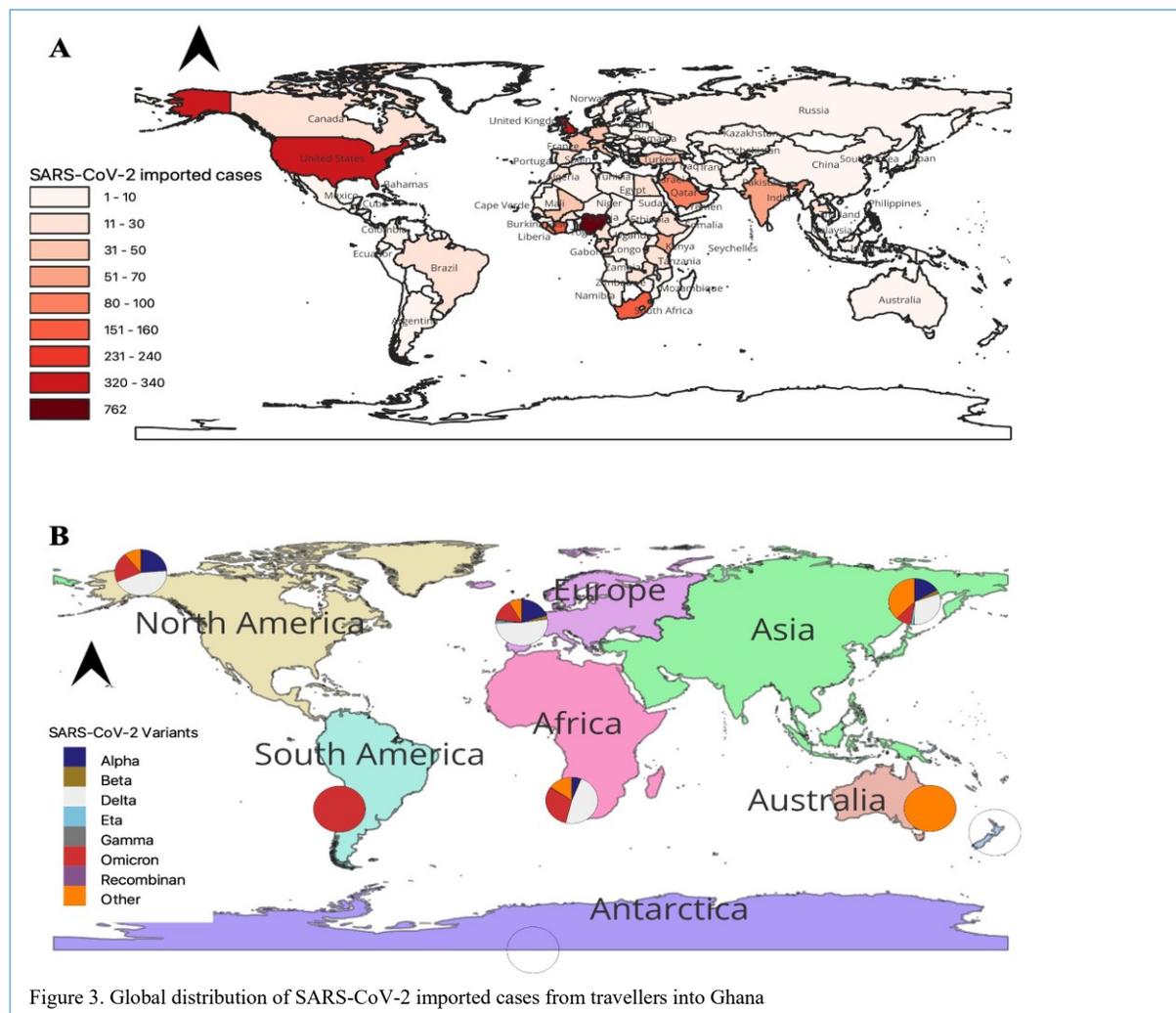


Figure 3. Global distribution of SARS-CoV-2 imported cases from travellers into Ghana

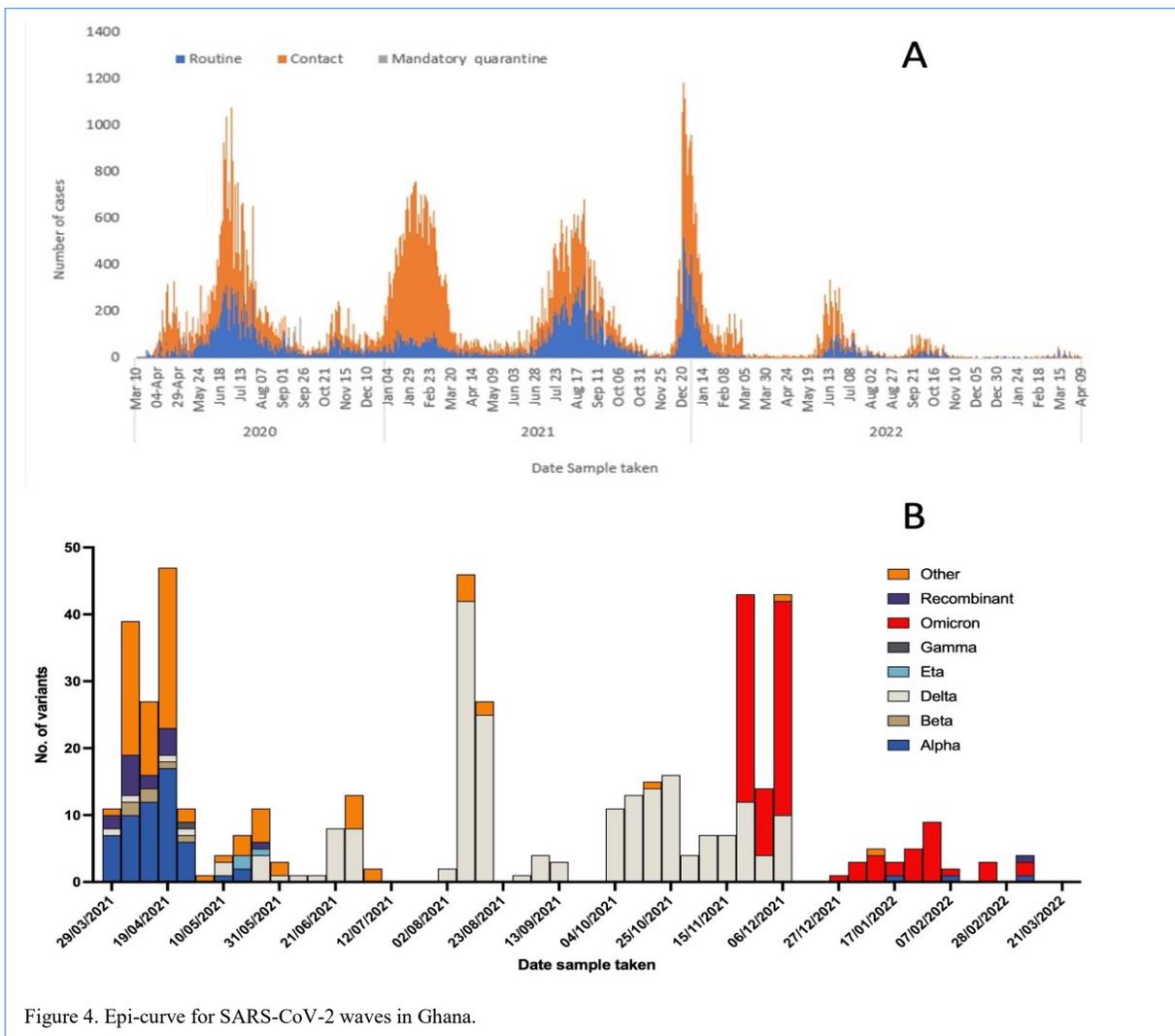


Figure 4. Epi-curve for SARS-CoV-2 waves in Ghana.

Eight distinct variants of SARS-CoV-2 were detected among travellers entering Ghana from January 2021 to March 2022 (i.e., the second to fourth wave of COVID-19 infections in Ghana). A few cases of beta, eta, gamma, and kappa variants were also detected during the period. Peaks of community transmission were detected from June to October 2021 and from December 2021 to March 2022. These were driven by the delta variant, its sub-lineages, and the Omicron variant.

DISCUSSION

Isolation has been shown to be a valuable tool for controlling the spread of disease, according to the extant literature, even before the COVID-19 pandemic [11]. International travel has been identified as a major contributor to the importation and spread of infectious diseases among countries [12]. Therefore, the benefits of isolation remain instrumental in containing contagious

diseases, as seen during the severe acute respiratory syndrome (SARS) pandemic in 2003 [11]. The swift decision by the GoG and the GHS to isolate inbound travellers who tested Ag+ for SARS-CoV-2 during the 2019 pandemic proved effective in preventing transmission into the community. This report describes observations from the mandatory isolation of SARS-CoV-2 Ag+ travellers arriving at the KIA Ghana at the height of the COVID-19 pandemic from January 2021 to March 2022.

A total of 3,331 inbound travellers testing Ag+ to SARS CoV-2 were placed under mandatory isolation during the study period. Our data showed that approximately 50% of the travellers were unvaccinated and had no hospitalisation record. It is known that young adults (aged <50 years) with no history of comorbidities present with asymptomatic disease and are known to be super-spreaders of COVID-19 [8]; hence, the isolation of all Ag+ persons. Our data confirmed these observations and hence, affirmed GoG's

position for mandatory isolation and testing of travellers. More importantly, vaccination may not have necessarily prevented infection or reinfection of SARS-CoV-2 but may have limited the severity of the disease as current vaccine effectiveness at preventing infection varied from 54% to 75% depending on the type of variants [7,13,14]. The GHS had embarked on active SARS-CoV-2 vaccination, resulting in 25,742,426 vaccine doses and 34% of the population fully vaccinated as of June 30, 2023 [5].

Furthermore, information on travel history indicated that 51% of SARS-CoV-2 cases arrived from Africa, while the remaining 49% arrived from other continents (Figure 3). This may be explained by the fact that during this period, many countries outside Africa were still under lockdown and strict travel restrictions. Further analysis showed that passengers arriving from Nigeria were in the majority (23%), with the UK (10%), US (9.8%), and UAE (9.7%) also showing significant presence.

Gene sequence data generated indicated that the delta and omicron variants of SARS-CoV-2 were first detected among travellers at the port of entry (KIA) before they appeared in the community, resulting in observed peaks in transmission from June to October 2021 and from December 2021 to March 2022 (Figure 4A & B). This finding was supported by studies that reported that the accumulation of specific mutations in the genes, particularly in the spike protein, of these variants enhances their transmissibility while reducing vaccine efficacy and the efficacy of antibody-based therapies [15,16]. On the contrary, another study among travellers that employed RAT in combination with rt-PCR reported a higher prevalence of SARS-CoV-2 by RAT than by rt-PCR; it was concluded that RAT was a suitable method for detecting COVID-19 among travellers during the outbreak [17]. Thus, it may be prudent to consider using both assays, RAT plus rt-PCR, for screening for infections during an outbreak to reduce the incidence of false negatives.

Conclusion

This study successfully screened and identified 3,331 SARS-CoV-2 cases among travellers arriving at Kotoka International Airport. Isolating these cases helped prevent potential transmission into the communities, and genomic sequencing revealed that the variants implicated in the global spread during the respective periods were consistent with those detected among the positive travellers over the study period. Additionally, our data showed that rapid antigen testing is suitable for detecting infectious diseases such as COVID-19. RATs can therefore serve as an important surveillance tool for infectious diseases of public health importance, particularly at points of entry. They provide a cost-effective, convenient, and rapid method for screening suspected cases, especially in outbreak settings where timely detection is critical for patient management and treatment.

DECLARATIONS

Ethical consideration

Ethical approval was not required for this study. Samples were collected at the height of the COVID-19 pandemic. This activity was considered an emergency surveillance activity by the Ghana government's COVID-19 Taskforce and the Ghana Health Service, and all travellers were sampled as an emergency measure against disease transmission.

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare no conflict of interest

Author contribution

WKA, IAA, FA-B, JOC, EKA, DDN, NKF-A, and DOL conceptualised the study. MAA-P, IAA, DDN, NKF-A, IA, IATG, NAAN, LB, LK, ID, and GMS developed the methodology. SON, YA-L, MAA-P, and IAA conducted data analysis and curation. IAA, MAA-P, WKA, FA-B, JOC, EKA, DDN, NKF-A, DOL, IA, and IATG carried out the investigation and provided resources. MAA-P, IAA, IA, and YA-L drafted the manuscript. All authors contributed to the review and editing. MAA-P, IAA, NAAN, DDN, NKF-A, and WKA supervised the study. All authors approved the final manuscript.

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Availability of data

Data is available upon request to the corresponding author

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