TRACKING SARS-COV-2 TRANSMISSION AND CO-INFECTION WITH OTHER ACUTE RESPIRATORY PATHOGENS USING A SENTINEL SURVEILLANCE SYSTEM IN RIFT VALLEY, KENYA

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Abstract

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has been the most significant public health challenge in over a century. SARS-COV-2 has infected over 765 million people worldwide, resulting in over 6.9 million deaths. This study aimed to detect community transmission of SARS-CoV-2 and monitor the co-circulation of SARS-CoV-2 with other acute respiratory pathogens in Rift Valley, Kenya. We conducted a cross-sectional active sentinel surveillance for the SARS-CoV-2 virus among patients with acute respiratory infections at four sites in Rift Valley from January 2022 to December 2022. 1271 patients of all ages presenting with influenza-like illness were recruited into the study. Nasopharyngeal swab specimens were screened using a multiplex RT–qPCR for SARS-CoV-2, Influenza A, Influenza B and RSV. Influenza A and RSV samples were subtyped, and all the SARS-CoV-2 positive samples were further screened for 12 viral and 7 bacterial respiratory pathogens. We had a prevalence of 13.93% SARS-CoV-2, Influenza A 5.7%, Influenza B 1.96% and 0.94%. Influenza A-H1pdm09 and RSV B were the most dominant circulating subtypes of Influenza A and RSV, respectively. The most common co-infecting pathogens were Streptococcus pneumoniae and Haemophilus influenzae, accounting for 16.4% and 10.7% of all the SARS-CoV-2 positive samples. Augmenting syndromic testing in ARI surveillance is crucial to inform evidence-based clinical and public health interventions.

INTRODUCTION

In late 2019 a cluster of pneumonia cases of unknown aetiology emerged in Wuhan, Hubei province, China. A novel coronavirus was reported to be the causative agent and officially named SARS-CoV-2 by the International Committee on Taxonomy of Viruses based on phylogenetic analysis. Within a short period, it had spread to nearly all countries in the world, attaining pandemic status with an estimated global case count of 765 million and 6.9 million deaths as of 24$^{\text{th}}$ May 2023 . To date, Kenya has recorded 343,073 SARS-CoV-2 cases and 5688 case fatalities.

Acute respiratory infections (ARIs) are a significant public health concern due to their widespread morbidity and mortality and their potential to cause pandemics. ARIs are transmitted primarily via large respiratory droplets, contact with surfaces contaminated by respiratory droplets, and aerosolized small respiratory droplets. Most patients with SARS-CoV-2 exhibit fever, sore throat and dry cough, and the less common symptoms are joint aches, rhinorrhoea, myalgia, dizziness, difficulty breathing, diarrhoea, chest pains, and nausea.

Early detection and monitoring of ARIs are crucial for controlling outbreaks and preventing their spread. Limited diagnostic capabilities, limited access to health care, and economic constraints frustrate early public health interventions. There are over 25 known viral and bacterial ARI pathogens. Patients with ARIs frequently present with symptoms indicative of disease but not specific enough to distinguish what makes them ill clinically. The lack of clear diagnosis results in delayed interventions. Sentinel surveillance maps the evolution of epidemics and provides evidence to inform control approaches in advance since hospital admissions and mortality indicators lag community transmission. It is an effective tool for monitoring the incidence of ARIs in a population and has been effectively deployed to monitor syndromic illnesses.

Understanding epidemiology and transmission dynamics is vital in providing timely and accurate information for evidence-based public health interventions. There have been several studies on co-infection of SARS-CoV-2 with other ARIs worldwide, with most focusing on co-infection with influenzas A and B. There is limited data about SARS-CoV-2 co-infection with other ARI pathogens in Kenya. Viral respiratory infections have been shown to predispose patients to secondary bacterial infections and alter host immunopathology leading to increased morbidity and mortality. Identifying pathogens co-infecting with SARS-CoV-2 is critical in developing clinical and public health measures to improve patient outcomes.

We aimed to address these evidence gaps by conducting an active sentinel surveillance study among patients meeting the case definition of suspected SARS-CoV-2 cases. We investigated the incidence of SARS-CoV-2, RSV, influenza A and influenza B after which we subtyped influenza-A and RSV-positive samples. Finally, we investigated the co-infection of all the SARS-CoV-2-positive samples with 19 viral and bacterial respiratory pathogens. This manuscript presents the results of an active sentinel surveillance program conducted in four sentinel sites in the Rift Valley region, Kenya. We describe the methodology used for data collection, demographic characteristics of the population under surveillance, incidence rates and trends of different ARIs.

METHODS

Study area

This study was conducted in Nakuru, Elgeyo Marakwet and Nandi Counties in the Rift Valley region. The sites in which the study was implemented include three district hospitals and one sub-district hospital (Figure 1). The sentinel sites were selected purposively due to the paucity of information regarding respiratory infections in these counties and their contributions to the healthcare burden in Kenya. A temperate climate characterizes the study location with an altitude ranging between 2000-2500m above sea level.

Case definition

The study adopted the following case definition for suspected SARS-CoV-2 cases: patients with one or more of the following symptoms within the last seven days; fever, cough, sore throat, and respiratory distress.

Study design and sample collection

We adopted a cross-sectional active sentinel surveillance study design. The study population included any person aged six months and above. At each sentinel site, the first five patients meeting the case definition of a suspected SARS-CoV-2 case were eligible for recruitment per day. Each participant signed a written informed consent form in either English, Kiswahili, or the local dialect; for minors, parents or guardians supplied the written consent. Children aged between 13 and 17 were asked to assent to participate in the study after consent had been sought from the caregiver. Socioepidemiological and clinicopathological patient data from each case was recorded using a standardized questionnaire including a unique identifier, demographics, symptoms, and pre-existing conditions onto ODK collect Version 4.4.

Specimen processing

Nasopharyngeal swab specimens were taken using FLOQswabs and placed in 2ml ENAT of Viral Transport Medium (VTM). The specimens were refrigerated at -20°C at the sentinel site and transported in a cold

chain to the Sample Management and Receiving Facility (SMRF), KEMRI, where they were stored at -80°C until they were ready for processing.

Detection of SARS-CoV-2, RSV, Influenza A and B

RNA was extracted using Zymo quick DNA/RNA extraction kit (Zymo Research, Irvine, USA) according to the manufacturer's protocols. Briefly, 400µl of each sample was extracted and eluted using 50µl of elution buffer. RT-qPCR was done using Allplex SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc, Seoul, South Korea) on Bio-Rad CFX96 (Bio-Rad Laboratories, Hercules, USA). This multiplex assay can detect SARS-CoV-2 (N gene, RdRP gene and S gene), Influenza A, Influenza B and Respiratory syncytial virus simultaneously. 5µl of extracted RNA was added to 15µl of mastermix for each reaction and amplification was performed at 50°C for 20 mins, 95°C for 15 mins, 2 cycles of 95°C for 10 secs, 60°C for 40 secs, 72°C for 20 secs, 41 cycles of 95°C for 10 secs followed by fluorescence detection at 60°C for 15 secs and 72°C for 10 secs. All runs were performed together with relevant controls to ensure validity. The results were exported to Excel (Office 365) and interpreted using Seegene Viewer (Seegene Inc, Seoul, South Korea).

Influenza A and RSV subtyping

Next, we subtyped all the Influenza A and RSV-positive samples using Seegene Allplex Respiratory Panel 1 (Seegene Inc, Seoul, South Korea). Allplex Respiratory Panel 1 is a multiplex assay for simultaneous detection and differentiation of three Influenza A subtypes (Influenza A-H1, Influenza A-H1pdm09, Influenza A-H3) and two RSV subtypes (RSV A and RSV B). Viral RNA was extracted from all Influenza A positive and RSV-positive specimens using Zymo quick DNA/RNA extraction kit (Zymo Research, Irvine, USA) according to the manufacturer's protocols. 8µl of extracted RNA was added to 17µl of mastermix, and RT-qPCR was done on Bio-Rad CFX96 (Bio-Rad Laboratories, USA). Amplification was performed at 50°C for 20 mins, 95°C for 15 mins, and 44 cycles of 95°C for 10 secs, followed by fluorescence detection at 60°C for 1 min and 72°C for 10 secs. All runs were performed together with relevant controls to ensure validity. The results were exported to Excel and interpreted using Seegene Viewer (Seegene Inc, Seoul, South Korea).

Detection of other respiratory pathogens

All the positive SARS-CoV-2 samples were then analyzed for co-infection with other respiratory pathogens using Seegene Allplex Respiratory Panels 2, 3, and 4 (Seegene Inc, Seoul, South Korea). These panels are multiplex kits for the identification of 12 viral and 7 bacterial pathogens. Respiratory panel 2 identifies Adenovirus, Enterovirus, Metapneumovirus and Parainfluenza virus types 1-4, whereas respiratory panel 3 is for identification of human bocavirus 1/2/3/4, human coronaviruses 229E, NL63, OC43 and human rhinovirus. Respiratory panel 4 is for identification of respiratory bacterial pathogens*Bordetella parapertussis, Bordetella pertussis, Chlamydophila pneumoniae, Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae* and *Streptococcuspneumoniae.* Nucleic acids were extracted from the specimens using Zymo quick DNA/RNA extraction kit (Zymo Research, Irvine, USA) according to the manufacturer's protocols. 8µl of extracted nucleic acids was added to 17µl of mastermix, and RT-qPCR was performed on Bio-Rad CFX96 (Bio-Rad Laboratories, USA). Amplification was performed at 50°C for 20 mins, 95°C for 15 mins, 44 cycles of 95°C for 10 secs, followed by fluorescence detection at 60°C for 1 min and 72°C for 10 secs. The cycling conditions were the same for all the panels. All runs were performed together with relevant controls to ensure validity. The results were exported to Excel and interpreted using Seegene Viewer (Seegene Inc, Seoul, South Korea).

Data analysis

The collected data were exported to Microsoft Excel (Office 365) and combined with the results laboratory results. Personal identification data were eliminated prior to statistical analysis. Descriptive statistics were performed for all the variables. Associations between SARS-CoV-2 and categorical variables were calculated using the Chi-squared test. All variables with p-values of [?]0.05 were considered statistically significant. The analysis was performed using R Studio.

Ethics statement

This study was approved by the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU No: KEMRI/SERU/CVR/012/4126). All respondents provided informed written consent.

RESULTS

In the four sentinel sites, 1271 individuals who met the predefined inclusion criteria were enrolled in the study. Among the participants, 510 (40.1%) were male, while 761 (59.9%) were female. The study cohort comprised 90.8% (N=1154) adults and 9.2% (N=117) minors. The median age of the study participants was 37 years, with an age range spanning from 3 to 98 years. The prevalence of SARS-CoV-2 during the study period was 13.93% (N=177), with higher rates among females (15.5%) compared to males (11.6%). Influenza A was the most common influenza subtype, with a prevalence of 5.7% (N=73), while influenza B was 1.96% (N=25). Influenza A was more prevalent among males (7.4%) compared to females (4.4%), whereas influenza B had similar rates among males (1.96%) and females (1.97%). There were RSV showed a prevalence of 0.94%, with similar rates among males (0.8%) and females (1.1%) (Table 1).

Among the positive samples for influenza A, the influenza A (H1N1)pdm 2009 subtype accounted for 50.6% (N=37), none were identified as Influenza A-H1 or Influenza A-H3, while 49.4% (N=36) could not be classified into specific subtypes. 50% (N=6) of the samples tested positive for RSV subtype B, and 8.3% (N=1) were identified as RSV subtype A while the rest could not be sub-typed (Supplementary Table 1).

SARS-CoV-2, Influenza A and influenza B were found to be circulating in all the sentinel sites, while RSV was present in three sites (Table 1). The prevalence varied widely across the sites, with Molo having the highest of SARS-CoV-2 and influenza A while Iten had the lowest in both cases. Olenguruone had the highest prevalence of influenza B. There were two instances of co-infection involving both influenza A and influenza B, one instance of co-infection with influenza A and RSV, and one case where all three pathogens (influenza A, influenza B, and RSV) were present simultaneously (Supplementary Table 2).

There were two major spikes in the incidence of SARS-CoV-2 and Influenza A. The spike in SARS-CoV-2 occurred in January and June-July due to the 5^{th} and the 6^{th} COVID-19 waves around the same time. The spike in Influenza A cases happened in June-July and September, reflecting seasonal patterns of influenza occurrence in Kenya.

The most common symptom in SARS-CoV-2 infected patients was rhinorrhoea (68.4%), then myalgia (53.7%), fatigue (54.1%), fever (52%) and anosmia (24.9%), whereas the most reported comorbidities were diabetes (5, 2.8%) and HIV (2, 1.1%) (Table 2). A summary of associations between various symptoms and comorbidities with SARS-CoV-2 status is shown in Table 2. Statistical analysis revealed a significant association between rhinorrhoea and SAR-CoV-2 (OR = 2.463, 95% CI = 1.731 - 3.523, $p < 0.001$). Influenza A patients commonly presented with symptoms of rhinorrhoea (66.7%) followed by fever (54.2%), myalgia (51.4%), and fatigue (48.6%), while those with influenza B commonly presented with rhinorrhoea(76%), fever(60%), fatigue(56%), and myalgia (56%). Among RSV-positive patients, myalgia (75%) and fever (58.3%) were the most frequent presenting symptoms (Table 2).

29.9% (N=53) of the SARS-CoV-2 positive samples were co-infected with one or more acute respiratory pathogens. The most detected co-infecting pathogen was *Streptococcus pneumoniae* 16.4% (N=29), followed by*Haemophilus influenzae* 10.7%, (N=19), HCoV OC43 5.9% (N=9), HCoV 229E 4% (N=7), Influenza A 2.3% (N=4), Influenza B 2.3% (N=4), human rhinovirus 2.3%(N=4) and HCoV NL63 1.1% (N=2). RSV, PIV 3, PIV 4, Metapneumovirus (MPV), Adenovirus (AdV), Enterovirus (HEV), Bocavirus 1/2/3/4 (HBoV), *Bordetella parapertussis (BPP), Bordetella pertussis (BP), Chlamydophila pneumoniae (CP), Legionella pneumophila (LP), and Mycoplasma pneumoniae (MP)* were however not detected as co-infecting with SARS-CoV-2 (Supplementary table 3).

Seventeen co-infection patterns with SARS-CoV-2 were found in this study (Table 4). There were 31 cases where one pathogen co-infecting with SARS-CoV-2, 18 cases of two pathogens, 2 cases of three pathogens, and 1 case where four pathogens co-infected with SARS-CoV-2. A high proportion of co-infection patterns was SARS-CoV-2 and *Streptococcus pneumoniae,* accounting for 22.6% (N=12) of all co-infections observed.

DISCUSSION

The timely detection and monitoring of ARIs is essential for understanding disease patterns and trends, developing appropriate prevention and control strategies, and informing public health decision-making. In this study, we implemented an active sentinel surveillance system for SARS-CoV-2 in four sites in the Rift Valley, focusing on detecting SARS-CoV-2 and co-infections with other acute respiratory pathogens. This study offers a glimpse of the respiratory pathogen landscape and the co-infection of SARS-CoV-2 with viral and bacterial ARIs in the Rift Valley, Kenya, providing valuable insights into disease burden, prevalence rates, and co-infection patterns.

As of December 2022, Kenya has had seven waves of SARS-CoV-2. The trend of SARS-CoV-2 infection corresponded to the national trend, with similar peaks during the fifth and sixth waves . This study captures a snapshot of the fifth wave on the decline. SARS-CoV-2 positivity of 13.93% rate is similar to that reported in Bukavu City in the Democratic Republic of Congo.

The trend of influenza A is consistent with the seasonality of influenza in Kenya, which corresponds to the winter season in the Southern hemisphere The prevalence of influenza A, 5.6%, influenza B, 1.96% and RSV, 0.95, is much lower than those from previous studies in Kenya. There is a significant dominance in the circulation of Influenza A (H1N1)pdm 2009 compared to what has been reported in previous studies suggesting a shift in the viral landscape and highlights, thus having a potential impact on the local disease burden. Whereas the study found a significant association between rhinorrhoea and SARS-CoV-2, it is inconsistent with previous studies, which found it to be a rarer symptom of SARS-CoV-2.

One of the most significant findings of this study was the high proportion of co-infections observed in patients with SARS-CoV-2 infection, with approximately 29.9% of the SARS-CoV-2 positive samples being co-infected with one or more acute respiratory pathogens.*Streptococcus pneumoniae* and *Haemophilus influenzae* were the most co-infecting ARI pathogens, which is similar to previous studies that identified these two pathogens as some of the most common co-infecting pathogens in ARIs . Bacterial aetiologies are often not investigated in most ARI cases as they usually present as secondary infections following a viral infection and require further diagnostic approaches, including culturing and antibiotic susceptibility. Co-infections, especially with bacterial pathogens, have been shown to complicate patients' clinical course, leading to poor disease outcomes. This study's findings thus underscore the importance of diagnosing bacterial ARI pathogens to address challenges arising from co-infections and prevent unnecessary antibiotic use, which could potentially lead to antimicrobial resistance.

Another notable finding was that human coronaviruses were the common viral aetiologies co-infected with SARS-COV-2. The circulation of human coronaviruses in Kenya has been reported before the emergence of SARS-CoV-2; hence little is understood about the clinical implications of co-infection . This highlights the importance of understanding potential interactions and cross-reactivity between different coronaviruses as this potentially impacts disease severity, immune response, clinical outcomes, and therapeutic strategies.

These findings thus demonstrate that other underlying pathologies warrant syndromic testing for evidence-based clinical interventions to improve patient outcomes. The main limitation of this study was that it was not possible to make further assessments of the impact of ARIs and co-infections on patient outcomes due to lack of information on clinical severity, hospitalization, recovery, and treatment. Additionally, the study relied on self-reported symptoms, which may not accurately reflect the clinical presentation of ARIs.

CONCLUSION

The findings from this study have important implications for public health policies and strategies to reduce the burden of ARIs in Kenya. It is the first study that reveals the co-infection of SARS-CoV-2 with other respiratory pathogens in Kenya, demonstrating that other underlying pathologies warrant syndromic testing for evidence-based public health interventions to minimize community impact. Sustained surveillance efforts of ARIs are necessary to monitor disease trends and inform public health decision-making.

REFERENCES

Tables

Table 1: Demographic characteristics of patients enrolled in the study.

Variables	No sampled	SARS-CoV-2	RSV	Influenza-A	Influenza-B
Gender					
Male	510 (40.1%)	59 (11.6%)	4 (0.8%)	38 (7.4%)	10 (1.96%)
Female	761 (59.9%)	118 (15.5%)	8 (1.1%)	34 (4.4%)	15 (1.97%)
Age					
Adults	1154 (90.8%)	159 (13.8%)	10 (0.9%)	65 (5.6%)	21 (1.8%)
Minors	117 (9.2%)	18 (15.2%)	2 (1.6%)	7 (5.9%)	4 (3.3%)
Sites					
Iten	479 (37.7%)	30 (6.2)	1 (0.21%)	5 (1%)	1 (0.2%)
Olenguruone	430 (33.8%)	69 (16.0%)	4 (0.93%)	27 (6.3%)	16 (3.7%)
Kapsabet	203 (16%)	21 (10.3%)	7 (3.4%)	22 (10.8%)	4 (1.97%)
Molo	159 (12.5%)	57 (35.85%)	0 (0%)	19 (11.95%)	4 (2.5%)
Total N (%)	**1271**	**177 (13.93%)**	**12 (0.94%)**	**73 (5.7%)**	**25 (1.96%)**

Table 2: Association between symptoms and comorbidities with SARS-CoV-2 Status. *** statistically significant

	SARS-CoV-2 Negative N =1094 (%)	SARS-CoV-2 positive N =177 (%)	O
Fever	506 (46.3)	92 (52)	1.
Anosmia	329 (30)	44 (24.9)	0.
Myalgia	543 (49.6)	95 (53.7)	1.
Rhinorrhoea	510 (46.7)	120(68.4)	2.
Fatigue	609 (55.7)	94 (53.1)	0.
Diarrhoea	35 (3.2)	7 (3.9)	1.
Chronic respiratory disease	2 (0.6)	1 (0.5)	3.
Diabetes	14 (1.2)	5 (2.8)	2.
HIV	5 (0.5)	2 (1.1)	2.

Table 3: Clinical characteristics of the Influenza A, Influenza B and RSV-positive patients

Variables	Influenza A positive N=72(%)	Influenza B positive N=25 (%)	RSV Positive N=12 (%)
Symptoms			
Fever	39 (54.2%)	15 (60%)	7 (58.3%)
Anosmia	20 (27.8%)	8 (32%)	5 (41.7%)
Myalgia	37 (51.4%)	14 (56%)	9 (75%)
Rhinorrhoea	48 (66.7%)	19 (76%)	4 (33.3%)
Fatigue	35 (48.6%)	14 (56%)	5 (41.7%)
Diarrhoea	1 (0.5%)	0 (0%)	0 (0%)
Comorbidities			
Diabetes	1 (0.5%)	0 (0%)	0 (0%)
Expositions			
Smoking	1 (0.5%)	0 (0%)	0 (0%)

Table 4: Co-infection patterns of SARS-CoV-2 with other acute respiratory pathogens

Virus types	**Co-infecting Pathogens**	**Cases**
SARS-CoV-2+Flu A	2	4
SARS-CoV-2+Flu B	2	4
SARS-CoV-2+PIV2	2	1
SARS-CoV-2+hCoV NL63	2	2
SARS-CoV-2+hCoV OC43	2	1
SARS-CoV-2+HRV	2	3
SARS-CoV-2+HI	2	4
SARS-CoV-2+SP	2	12
SARS-CoV-2+PIV1+ HI	3	1
SARS-CoV-2+PIV1+ HI+SP	4	1
SARS-CoV-2+hCoV 229E+HI	3	4
SARS-CoV-2+hCoV 229E+hCoV OC43+HI+SP	5	2
SARS-CoV-2+hCoV 229E+SP	3	1
SARS-CoV-2+hCoV OC43+SP	3	6
SARS-CoV-2+HRV+SP+HI	4	1
SARS-CoV-2+HI+SP	3	6

Supplementary Table 1: Distribution of Influenza A and RSV Subtypes

Influenza A subtype	N=73 (%)	**RSV subtype**	N=12 (%)
Influenza A-H1	0	RSV A	1 (8.3%)
Influenza A-H1pdm09	37(50.7%)	RSV B	6 (50%)
Influenza A-H3	0	Un-subtyped	5 (42.7%)
Un-subtyped	36(49.3%)		

Supplementary Table 2: Co-infection Cases of Influenza A, Influenza B, and RSV

Co-infections	**Cases**
Influenza A + Influenza B	2
Influenza A + RSV	1
Influenza A + Influenza B +RSV	1

Supplementary Table 3: Distribution of the co-infecting pathogens in the study population

Co-infecting Pathogens	**Cases, N=177 (%)**
Influenza A (Flu A)	4 (2.2%)
Influenza B (Flu B)	4 (2.2%)
Respiratory syncytial virus (RSV)	0 (0%)
Parainfluenza virus 1 (PIV 1)	2 (1.1%)
Parainfluenza virus 2 (PIV 2)	1 (0.57%)
Parainfluenza virus 3 (PIV 3)	0 (0%)
Parainfluenza virus 4 (PIV 4)	0 (0%)
Metapneumovirus (MPV)	0 (0%)

Co-infecting Pathogens	Cases, N=177 (%)
Adenovirus (AdV)	0 (0%)
Enterovirus (HEV)	0 (0%)
Bocavirus 1/2/3/4 (HBoV)	0 (0%)
Coronavirus 229E (hCoV 229E)	7 (4%)
Coronavirus OC43 (hCoV OC43)	9 (5.1%)
Coronavirus NL63 (hCoV NL63)	2 (1.1%)
Human Rhinovirus (HRV)	4 (2.2%)
Bordetella parapertussis (BPP)	0 (0%)
Bordetella pertussis (BP)	0 (0%)
Chlamydophila pneumoniae (CP)	0 (0%)
Haemophilus influenzae (HI)	19 (10.7%)
Legionella pneumophila (LP)	0 (0%)
Mycoplasma pneumoniae (MP)	0 (0%)
Streptococcus pneumoniae (SP)	29 (16.4%)

Figure Legends

Figure 1: Map Showing the locations of the sentinel sites.

Figure 2: SARS-CoV-2, Influenza and RSV cases weekly trends.