

Original Research Article

SARS-CoV-2 recurrence and probable reinfection: outcome of a descriptive surveillance in a Nigerian tertiary hospital

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ABSTRACT

Background: The reports of SARS-CoV-2 reinfection have increased. This stimulates the need for surveillance in diverse populations to establish the extent of reinfections and the challenges to diagnosis.

Methods: A retrospective descriptive survey aimed at identifying probable SARS-CoV-2 reinfections using established criteria and proposed definitions was performed at a tertiary hospital in South-South, Nigeria.

Results: The study found two cases for evaluation of reinfection. One case was identified as probable reinfection, pending the outcome of gene sequencing, while the second case was categorized as recurrence. The limited access to routine genetic sequencing for confirmation of reinfection was identified as a key challenge.

Conclusions: Probable SARS-CoV-2 reinfections occur in Nigeria. Systematic surveillance of SARS-CoV-2 testing at the state and country-level is advocated to have a more accurate estimate of the burden of reinfections in the country. Access to genetic sequencing should be scaled up in Nigeria.

Keywords: SARS-CoV-2, COVID-19, Reinfection, Recurrence, Genome sequencing, Nigeria

INTRODUCTION

The novel SARS-CoV-2 pandemic has presented many challenges to control efforts. These challenges which are driven by the novel and dynamic nature of the disease have been characterised by progressing evidence on transmission dynamics, symptomatology and immune response.¹

The ideal goal of life long or long term immunity in patients post SARS-CoV-2 infection has been a subject of interest, following the pandemic.² The motivation for this ideal was the benefit of long term immunity in promoting sustained pandemic control through the attainment of

herd immunity and the prevention of reinfection, in contrast to short term immunity.

An analysis by Edridge et al on the immune response and reinfection to other coronaviruses with modelled extrapolation and predictions to SARS-CoV-2, revealed that a short term duration of protective immunity, high frequency of reinfections at 12 months and substantial reduction in antibody levels at 6 months seen with other coronaviruses may be expected with SAR-CoV-2.³ The implications of the findings by Edridge et al on the public health response to SARS-CoV-2 include significant risk of reinfections, limitations to the attainment of herd immunity and the futility of the immunity passport

concept for those with the previous disease, therefore, hindering early containment of the pandemic.³

The issue of reinfection from SARS-CoV-2 had initially been a subject of debate.^{4,5} This controversy was promoted by the lack of evidence of culture-based documentation of a new infection following clearance of the preceding infection or evidence of reinfection with a molecularly distinct form of the same virus; despite reports of reoccurrence of SARS-CoV-2 positivity and symptoms of COVID-19 in patients.^{2,5-9}

The report of confirmed reinfection with SARS-CoV-2 in a male in Hong Kong four and half months after initial infection, pushed the issue of SARS-CoV-2 reinfection beyond the myth to reality.^{10,11} Further reports of cases in the USA, Ecuador, Belgium, Netherlands, India, Turkey and Qatar as shown in Table 1 have erased existing doubts about the possibility of SARS-CoV-2 reinfection.^{6,12-20} There are currently 65 cases of confirmed reinfections globally across 17 countries as of 20 March 2021.²¹

Table 1: Characteristics of cited reports of SARS-CoV-2 reinfection.

Author	Citation	Location	Dates of infection	Age	Gender	First episode	Interval	Second episode	Publication
To et al	11	Hong Kong	26 March 2020/15 August 2020	33	Male	Symptomatic	142	Asymptomatic	Peer reviewed
Tillett et al	12	Nevada, USA	18 April 2020/5 June 2020	25	Male	Symptomatic	48	Symptomatic with hospitalisation, more severe	Peer reviewed
Larson et al	13	Virginia, USA	21 March 2020/24 May 2020	42	Male	Symptomatic	51	Symptomatic, more severe	Peer reviewed
Van Elslan de et al	15	Belgium	9 March 2020/10 June 2020	52	Female	Symptomatic/moderate	93	Symptomatic/Milder	Peer reviewed
Mulder et al	16	Netherlands		89	Female	Symptomatic	59	Symptomatic, more severe	Peer reviewed
Prado-Vivar et al	14	Ecuador	16 May 2020/20 July 2020	46	Male	Symptomatic	63	Symptomatic	Peer reviewed
Gupta et al	17	India	5 May 2020/21 August 2020	25	Male	Asymptomatic	108	Asymptomatic	Peer reviewed
		India	17 May 2020/5 September 2020	28	Female	Asymptomatic	111	Asymptomatic	
Ozars et al	18	Turkey	9 April 2020/4 August 2020	23	Female	Symptomatic	116	Symptomatic	Peer reviewed
Selhorts et al	19	Belgium	March 2020/Sept 2020	39	Female	Symptomatic/moderate	185	Symptomatic/mild	Peer reviewed
Abu Radda et al	20	Qatar	12 Jun 20/8 Aug 20	40-44	Female	Not hospitalised	56	Not hospitalised	Peer reviewed
	20	Qatar	3 Jun 2020/7 Aug 2020	40-44	Male	Not hospitalised	67	Not hospitalised	Peer reviewed
	20	Qatar	25 April/10 Jun 2020	25-29	Male	Not hospitalised	46	Not hospitalised	Peer reviewed
	20	Qatar	26 April/6 July 2020	40-44	Male	Not hospitalised	70	Not hospitalised	Peer reviewed
	20	Qatar	2 May/29 Jul 2020	45-49	Female	Not hospitalised	88	Not hospitalised	Peer reviewed
	20	Qatar	26 April/20	25-	Male	Not	55	Not hospitalised	Peer

Continued.

Author	Citation	Location	Dates of infection	Age	Gender	First episode	Interval	Second episode	Publication
			Jun 2020	29		hospitalised			reviewed
Fageeh et al	28	Saudi Arabia	10 June 2020/Sept 2020	24	Male	Symptomatic /mild	103	Symptomatic/less mild	Peer reviewed

Though many patients may be seen with positive SARS-CoV-2 RT-PCR tests and recurrence of symptoms at different points in time, the diagnosis or confirmation of reinfection is relatively uncommon, as various factors may influence such presentations.^{6,7,22} Also the determination of reinfection has to be proved beyond any doubts by establishing proof of replicating virus by cell culture, the detection of sub-genomic (RNA) at different time-points and the confirmation of infection with two different phylogenetic strains.^{6,7}

The diagnosis of reinfections has also shown the variability of immunity arising from SARS-CoV-2 infections. While it was thought that reinfection was unlikely within 90 days, reports of reinfection occurring below 90 days of the first infection have been documented.^{7,12-14,17,22}

It has been documented that the diagnosis of reinfection and interpretation of SARS-CoV-2 PCR tests can be challenging as patients may have positive RT-PCR days or weeks after recovery and previous negative results.²³⁻²⁵ It is also known that detection of RNA in any sample, does not necessarily mean the presence of the complete virus in the host or an active infection. A positive RT-PCR test also does not certainly imply SARS-CoV-2 viability, even if the genome is sequenced.^{5,24}

Other factors that may explain false positive of presumed reinfections include the recurrence of disease and delayed viral clearance associated with dynamic SARS-CoV-2 level, that result in wavering positive and negative tests.⁵

Inaccurate testing has also been found to result in false positives.⁵ This has been corroborated by Xiao et al who found that 21.4% of patients had a positive test using RT-PCR test even after two consecutive negative results, indicating that false positives caused by prolonged nucleic acid conversion may be responsible and masquerade as probable reinfections.²⁴

Additional challenges to the diagnosis of reinfection, globally and especially in low resource settings include logistical and capacity issues, such as the inability to bank samples from primary and repeat infections for the performance of viral genome analysis as well as the lack of routine genomic sequencing and viral cultures facilities.^{22,26}

These factors, therefore, make it more challenging to diagnose reinfections in low resource settings. These

observations have prompted calls for the review of diagnostic criteria to promote easy identification of reinfection.^{6,7}

Yahav et al have therefore proposed standardised definitions for reinfections, relapsed infections and recurrence of positive (re-positive) nucleic acid detection which can be applied in the setting of microbiologically confirmed reinfection, clinical reinfection and epidemiological reinfection aimed at improving the identification of reinfection.⁷

With the growing number of cases of reinfections with SARS-CoV-2, it is evident that many cases of reinfections may be missed especially in asymptomatic persons.¹¹⁻²⁰ Other contributory factors include the lack of routine surveillance for the identification of reinfection and the logistic challenges mentioned earlier.²⁶

Therefore the need for surveillance on SARS-CoV-2 reinfections in varied populations is useful, to evaluate the rate of occurrence and the contextual impact in areas with limited access to routine viral cultures and genome sequencing.

The objective of the survey was to evaluate the occurrence of SARS-CoV-2 reinfections among attendees at a tertiary hospital offering COVID-19 response services in South-South Nigeria.

METHODS

Study location

The study was conducted in Rivers state, one of Nigeria's 36 states located in South-South, Nigeria. The state ranks within the top 7 in the number of COVID-19 cases in the country with 6,235 cases as stated by the Nigerian centre for disease control (NCDC) as of 19 February 2021.²⁷

Study design

This study was a retrospective descriptive analysis of the records of patients who presented to University of Port Harcourt Teaching Hospital, Port Harcourt for COVID-19 screening and diagnosis with or without symptoms, based on SARS-CoV-2 Real-Time PCR screening from 15th May 2020 to 31st December 2020. The data of patients diagnosed with COVID-19 with or without symptoms on more than one occasion with a positive

SARS-CoV-2 RT PCR test was then evaluated for probable reinfection.

The patients' data were evaluated for the probability of reinfection based on the criteria established by the European centre for disease control as shown in Figure 1 and the proposed definitions for evaluation and diagnosis of reinfections by Yahav et al shown in Table 2 and Table 3.^{6,7}

Key parameters which were extracted for analysis as recommended by the European CDC and within the limits of available resources included epidemiological information (age, sex and occupation), results from investigations of possible exposure, clinical information (presence and severity of symptoms (if any) in both episodes), (clinical course of each episode, time-to-

detection and recovery time), (extent of symptom resolution (if any) between the two episodes), (time elapsed between the first episode and the suspected second episode of infection), information on testing by test result and specimen.⁶ Other variables were testing methodology, the timing of testing, place and reason of testing (e.g. screening border, primary care, hospital emergency or inpatient hospitalization), specimen type (e.g. respiratory, saliva), for RT-PCR results: cycle threshold value (Ct-value), immune assessment tests (duration/persistence, type and titres of antibodies (range), detection of neutralizing antibodies, if available: paired serological specimens from both the first (day 0 and 14) and the second infection (day 0 and 7, possibly also day 14), T-cell immunity and biomarkers such as CD40L, virus culture from multiple specimen types and comparative genomic analyses.

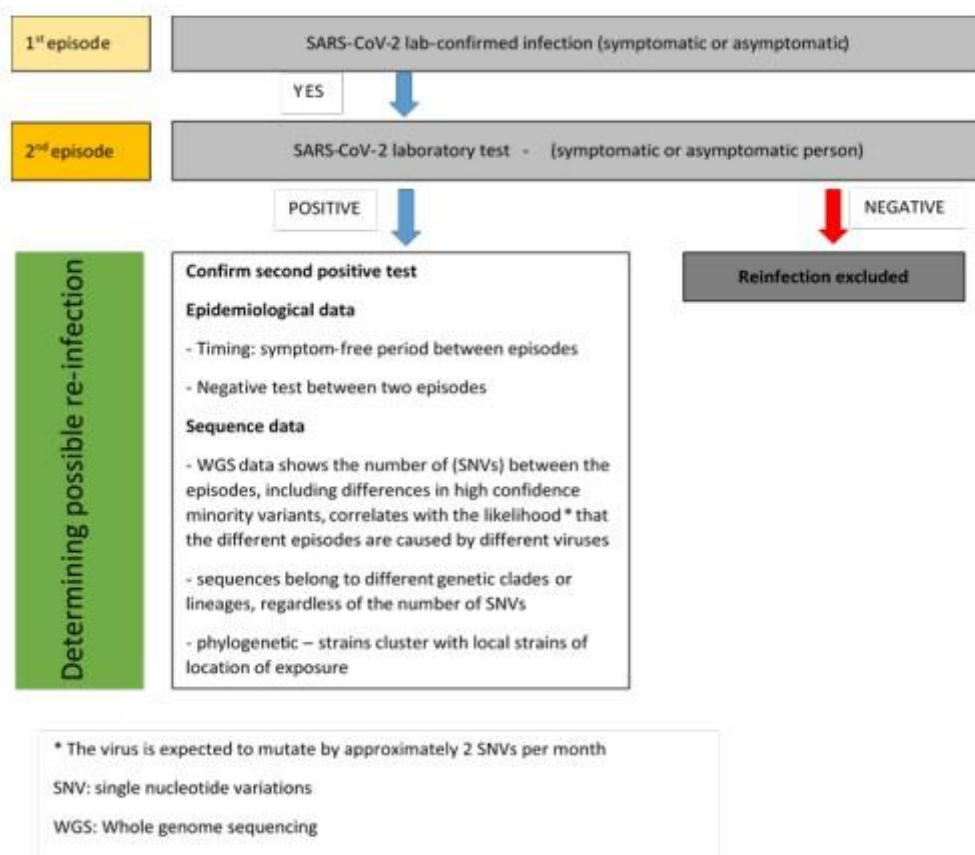


Figure 1: Assessment of reinfection recommended by European CDC.⁶

Table 2: Proposed definition for COVID-19 reinfections, relapse and re-positivity.⁷

Variables	Confirmed reinfection	Clinical reinfection	Epidemiological reinfection	Relapse/ reactivation	Repositivity
Clinical symptoms	Characteristic; clinical symptoms ^a	Characteristic; clinical symptoms ^a	Asymptomatic/ symptomatic	Characteristic; clinical symptoms ^a	Asymptomatic
PCR	Positive	Positive	Positive	Positive	Positive
Viral culture (should one be performed)	Positive	Positive	Positive	Positive	Negative

Continued.

Variables	Confirmed reinfection	Clinical reinfection	Epidemiological reinfection	Relapse/ reactivation	Repositivity
Time frame from original infection	>90 days ^b			<90 days	<90 days
Isolation measures	Recommended	Recommended	Recommended	Should be considered	Not recommended
Additional findings	Viral RNA sequencing from both episodes show different strains	Epidemiologic risk factor (known exposure or outbreak setting), no other cause	Epidemiologic risk factor (known exposure or outbreak setting)	No new exposure, area of low community spread	-

^aClinical manifestations characteristic of coronavirus disease 2019 (COVID-19); ^bcould be considered in the event of under 90 days if recovery proven by two consecutive negative PCR tests and current known COVID-19 exposure.

Table 3: Proposal definitions for evaluation and diagnosis of reinfections by Yahav et al.⁷

Evaluations	Diagnosis of reinfection
SARS-CoV-2 reinfection	A confirmed diagnosis of reinfection will require:
	confirmation of a true first episode, description of the viral load of the first episode is necessary (Ct values >35 might imply possible contamination rather than true infection); re-testing of the original specimen is indicated whenever possible.
	Proof of reinfection with two positive SARS-CoV-2 RT-PCR tests with Ct <35 (or proof of replicating virus by cell culture or detection of subgenomic RNA) at different time-points; plus.
	Confirmation of infection with two different phylogenetic strains by high-throughput sequencing, corresponding to local epidemiology (proof of two distinct virus variants with any sequence variation between the two episodes); plus.
	At least one, and ideally two, negative RT-PCR tests, on two different specimens collected between the first and second episodes, should be documented.
	For clinical practice: reinfection may be defined as
	clinical recurrence of symptoms compatible with COVID-19, accompanied by positive PCR test (Ct <35), more than 90 days after the onset of the primary infection, supported by close-contact exposure or outbreak settings and no evidence of another cause of infection.
	In the presence of epidemiological risk factors (significant exposure), reinfection should be considered during the first 90 days, if clinical symptoms of the first episode resolved and two PCR tests were negative before the new episode; viral culture, if collected, is expected to be positive.
COVID-19 relapse	Serology does not play a factor in the reinfection definition and could be either positive or negative after the first infection; a negative serology indicates either the absence of a potent detectable immune response or antibody waning; positive serology indicates that neutralizing antibody titres were not sufficient to eliminate the viral inoculum or that the infecting virus is substantially different to the first infection and is not recognized by the antibodies.
	For epidemiological purposes: reinfection could be defined as
	any positive RT-PCR test (Ct values <35) more than 90 days from the first episode, regardless of symptoms; since confirmation by genotypic assays is time and resource consuming, any case of suspected reinfection should be considered for isolation.
	Regarding RT-PCR re-positivity within 90 days, further studies performing genotypic assays of first- and second-episode specimens are needed to define reinfection during this period.
COVID-19 relapse	COVID-19 relapse, recrudescence, recurrence or reactivation could be defined as
	clinical recurrence of symptoms compatible with COVID-19 accompanied by positive/persisting RT-PCR within 90 days of primary infection and supported by the absence of epidemiological exposure or another cause of the illness.
	A viral culture may be positive.
	Demonstration of same strain by whole-genome sequencing could definitively differentiate this entity from reinfection (confirmed relapse).

SARS-CoV-2 PCR testing methodology

Sample collection

All samples were collected at the University of Port Harcourt Teaching Hospital, Port Harcourt, COVID-19 sample collection centre by trained healthcare workers in compliance with standard infection prevention and control protocols in line with the NCDC sample collection protocols.²⁸ Swab samples were collected from both the nasopharynx and oropharynx with separate swabs. Swabs were gently inserted through the nostril to a distance equivalent to the outer opening of the ear canal and gently rubbed for several seconds to absorb the secretions. For oropharyngeal samples, the tonsillar pillars were swabbed. The two swabs were then placed in a single tube containing 300 µl of viral transport medium to maximize the sensitivity of the RT-PCR test and

Amplification was by hydrolysis probe measurement systems using bio molecular systems (BMS) and gene loci of interest were nucleocapsid protein (N-gene), E-gene, RdRp-gene and open reading frame 1b (ORF1b) respectively. The following one-step PCR protocol was used: one cycle at 45°C for 10 minutes and 95°C for 3 minutes, followed by 45 cycles at 95°C for 15 seconds and 58°C for 30 seconds with single-point fluorescence detection at 58°C. The detection limit of the RdRp RT-PCR assays was approximately 1.0×10^3 copies/ml. Crossing point (Cp) values were used to determine SARS-CoV-2. Following colour difference compensation, amplified viral fragments were detected in the FAM, VIC/JOE and TEXAS RED and CY5 fluorescence channels. A Ct-value of the test sample in the FAM and VIC channels sequences each <40, with an obvious amplification curve, was defined as positive for SARS-CoV-2. A test with a Ct-value of ≤40 in only one channel between the FAM and VIC without an amplification curve in the other channel was defined as indeterminate and retested. If the result of the retest is consistent with the original it is determined as positive for SARS-CoV-2 while a negative retest is considered as negative. Test with Ct-value >40 was defined as negative.

Limitations

Routine evaluation for gene sequencing, phenotype variability and viral cultures are not available in the facility. Antibody test was also not applied on all patients in the facility for SARS-CoV-2 evaluation. However, antibody evaluation was not mandatory for the diagnosis of reinfection.⁷ Patients with symptoms are most likely to report and get diagnosed with reinfections, asymptomatic patients are therefore likely to be missed.

Ethical considerations

The ethical approval was obtained from the research ethics committee before the commencement of the study. Confidentiality was maintained by the removal of patient identifiers from the dataset and ensuring that only

minimize the use of resources. Samples were moved to the reference laboratory under cold chain conditions for subsequent processing and testing.

RNA extraction and RT-PCR testing for SARS-CoV-2 genes

Viral nucleic acid extraction and RT-PCR RNA was extracted in a type II biosafety chamber with HEPA filters, at the molecular virology laboratory of the NCDC certified laboratory. RNA extraction was done from samples arranged in pairs using a viral RNA extraction kit and the Ex2400 extraction system (Liferiver) or QIA amp viral RNA mini kit (Qiagen). A sample volume of 300 µl was used for RNA extraction and the elution volume was 50 µl. RT-PCR for SARS-CoV-2 detection was done using Daagene and GeneFinder COVID-19 plus realamp kit according to the manufacturer's protocol.

researchers involved in this study had access to the extracted data.

Data analysis and result presentation

Simple descriptive and comparative analysis using comparator tables with features of the criteria stated above was utilised. The proportion of cases that met the criteria for reinfection was evaluated using the number of clients with SARS-CoV-2 positivity as the denominator. Outcomes were presented using tables and figures as appropriate.

RESULTS

A total of 589 were diagnosed with COVID-19 based on an initial positive SARS-CoV-2 RT-PCR test. Two patients (0.34%) who met the criteria of repeated positive tests and different episodes of illness were evaluated for probable reinfection, with features as displayed in Table 4. The patients were both healthcare workers, a female nurse and a male doctor.

The cases were subjected to diagnostic criteria evaluation by ECDC and proposed definitions by Yahav et al for concordance with diagnostic algorithm and definitions as shown in Table 4.^{6,7} One patient was categorized as COVID-19 recurrence or repositivity while the other patient was categorized as probable reinfection as shown in Table 4 resulting in a probable reinfection rate of (0.17%).

COVID-19 recurrence or repositivity

A 32-year-old female with episodes 90 days apart (1 June 2020 to 1 September 2020), first episode with positive SARS-CoV-2 PCR, second episode positive SARS-CoV-2 PCR not confirmed on repeat testing, no negative RT-PCR test in between two episodes PCR Ct-value was <35 in the first episode and >35 in the second episode (Table 5).

Probable COVID-19 reinfection

37 year old male with episode 197 days apart (4 June 2020 to 18 December 2020), first episode with positive

SARS-Cov-2 PCR, second episode positive SARS-CoV-2 PCR positivity confirmed on repeat testing, consistent PCR Ct-values <35, negative RT-PCR test in between two episodes (Table 5).

Table 4: Epidemiologic and clinical features of patients with probable reinfection.

Variables	Patient 1		Patient 2	
	Initial COVID-19 disease	Recurrence/reinfection	Initial COVID-19 disease	Recurrence/reinfection
Age	32		37	
Gender	Female		Male	
Occupation	Healthcare worker		Healthcare worker	
History of contact	Yes	Unknown	Yes	Yes
Date of contact	24 May 2020	Unknown	1 June 2020	13 December 2020
Source of contact	Patient in hospital	Unknown	Patient in hospital	Patient in hospital
Reason for screening	Contact and symptoms	Symptoms	Contact and symptoms	Contact and symptoms
Date of the first symptom	28 May 2020	30 September 2020	4 June 2020	18 December 2020
Symptoms	Anosmia, low-grade fever	Sore throat	Sore throat, cough, low-grade fever.	High-grade fever, cough, anosmia, arthralgia, fatigue
Clinical parameters	Pr=82 b/min, spo ₂ =98%, Rr=22 /min, temp=37.2°C, Bp=110/60 mmhg	Pr=88 b/min, spo ₂ =98%, Rr=18 /min, temp=37.5°C, Bp=110/63 mmhg	Pr=82 b/min, spo ₂ =98%, Rr=22 /min, temp=37.2°C, Bp=110/60 mmhg	Pr=118 b/m, spo ₂ =98%, Rr=20 /min, temp=38.7°C, bp=130/70 mmhg
Comorbidity	None	None	Diabetes	Diabetes
Disease category	Symptomatic/mild	Symptomatic/mild	Symptomatic/mild	Symptomatic/mild
Specimen	Nasopharyngeal and oropharyngeal swap	Nasopharyngeal and oropharyngeal swap	Nasopharyngeal and oropharyngeal swap	Nasopharyngeal and oropharyngeal swap
Dates of SARS-COV-2 RT-PCR positive test	01 June 2020	1 September 2020	6 June 2020	23 December 2020
Ct-value n gene	18.37	35.48	23.87	26.42
Ct-value rdrp	18.86	37.48	23.92	26.46
Confirmation of second test result		Not confirmed positive		Confirmed positive
The interval between episodes (days)	90		197	
Date of repeat sars-cov-2 rt pcr negative test	Discharged with a time frame (14 days from 1 test)	5 September 2020	22 June 2020	30 December 2020
Treatment model	Hospitalisation	Home-based care	Home-based care	Home-based care
Date of discharge	15 June 2020	6 September 2020	23 June 2020	30 December 2020

Table 5: Evaluation of patient features based of diagnostic algorithms and definitions.

Subject descriptions		Patient 1: 32 year old female, 90 days interval between episodes	Patient 2: 37 year old make, 197 days between episodes
European CDC algorithm variables (EDCD)			
First episode	SAR-CoV-2 lab confirmed infection (symptomatic or asymptomatic)	Yes	Yes
Second episode	SAR-CoV-2 lab confirmed infection (symptomatic or asymptomatic)	No	Yes
Outcome		Reinfection excluded	Probable reinfection
Epidemiological data	Timing and symptom-free period between episode	Yes	Yes
	Negative test between episode	No (discharged based on time)	Yes
	Genome sequencing outcome	Awaited	Awaited
Outcome		Reinfection excluded (possibly a relapse, reoccurrence or reactivation)	Probable reinfection
Proposed definitions criteria (Yahad et al)			
A confirmed diagnosis of reinfection will require Confirmation of a true first episode description of the viral load of the first episode is necessary (Ct values >35 might imply possible contamination rather than true infection); re-testing of the original specimen is indicated whenever possible.		Yes	Yes
Proof of reinfection with two positive SARS-CoV-2 RT-PCR tests with Ct <35 (or proof of replicating virus by cell culture or detection of sub-genomic RNA) at different time-points; plus:		No	Yes
Confirmation of infection with two different phylogenetic strains by high-throughput sequencing, corresponding to local epidemiology (proof of two distinct virus variants with any sequence variation between the two episodes); plus:		NA	NA
At least one, and ideally two, negative RT-PCR tests, on two different specimens collected between the first and second episodes, should be documented		No	Yes
Outcome/interpretation		Reinfection excluded (possibly a relapse, recurrence or reactivation)	Probable reinfection

DISCUSSION

The subject of SARS-CoV-2 reinfection is no longer a matter of doubt with the increasing number of reports of SARS-CoV-2 reinfection, suggesting that the phenomenon may be more common than currently perceived.¹¹⁻¹⁹ The analysis of reported cases also shows that cases of reinfection had occurred before the confirmation of the first reported case in Hong Kong.^{12,13,15} These findings highlight the relevance of systematic surveillance programs targeted at the identification of reinfection with SARS-CoV-2 in various settings.

The challenges that impeded early identification of reinfections include the uncertainty of SARS-CoV-2 PCR interpretations arising from reoccurrence of disease, delayed viral clearance with wavering positive and negative tests and persisting positive viral tests which do not mean ongoing disease.^{5,22} These challenges, therefore, make the distinction and identification of separate disease episodes through relatively higher technological procedures such as the use of viral cultures and genome sequencing a necessity. However, the ease of applicability and access to these services may then impede the identification of reinfections in areas of limited resource.^{6,7,19}

This study found two patients (healthcare workers) with symptomatic episodes of suspected SARS-CoV-2 infection diagnosed with RT-PCR at 90 and 197 days apart. Further evaluation using the algorithms and proposed definitions by ECDC and Yahav et al documented one as a case of recurrence and while the other was a probable re-infection.^{6,7} The outcome of this case of probable reinfection will be determined by the outcome of genomic sequencing at national and supranational laboratories. This study highlights some challenges in the diagnosis of reinfections and interpretation of repeat positive RT-PCR for SARS-CoV-2 tests. The issues around the immune response after COVID-19 disease is also highlighted.

The prevalence of probable reinfection in this survey was (0.17%), this figure is higher compared with the finding of Abu-Raddad et al in Qatar who reported a reinfection risk of 0.02% from an analysis of centralized and standardized national SARS-CoV-2 testing and hospitalization database.²⁰ A report on reinfection status from the USA, estimates the prevalence of possible reinfections in Colorado at 0.1% which approximates the findings of this survey.^{21,26}

Though the rates of possible reinfections and confirmed reinfections are seemingly low, the extrapolated numbers of reinfection cases based on these rates will certainly be far more than the total cases of reinfections reported globally. Also, the investigations of all probable cases of reinfections which are limited by lack or absence of samples and resulting lack of genetic materials and the

difficulty in accessing genetic sequencing are likely to increase the numbers of confirmed reinfections.

Healthcare workers are at risk of consistent exposure and reinfection with SARS-CoV-2. The two subjects evaluated for reinfection in this survey were healthcare workers. Healthcare workers comprise a significant proportion of documented reinfection cases as shown by Larson et al in the USA, Selhorst et al in Belgium, Gupta et al in India and Ozaras et al in Turkey.^{13,16,18,19} Also, another report of confirmed reinfection in a dental student in Saudi Arabia and case reports of repeat COVID-19 episodes in health workers from Brazil show the risks of reinfection among health workers.²⁹⁻³¹ It is worthy of note that some of these diagnoses have been made in asymptomatic healthcare workers.¹⁸ This underscores the need for routine surveillance testing and evaluation for probable reinfections.

The diagnosis of patients with reinfections is most likely when the two episodes of symptomatic illness occur as seen in the patients in this survey and other case reports.¹²⁻¹⁶ The probability of missing out on cases of asymptomatic reinfections is therefore high. The occurrence of reinfections in asymptomatic patients has been shown by Gupta et al in two health workers.¹⁷ Also, the first reported case of reinfection from Hong Kong was associated with an asymptomatic second disease episode.¹¹ These results show that in the absence of routine surveillance especially in risk settings like healthcare institutions most asymptomatic cases of reinfections may be missed.

The timing from the first disease was initially used as criteria to exclude reinfections with assertions that reinfections were unlikely before 90 days of the first disease.^{7,19} In this analysis of two patients, the case with a report at 90 days was least likely to be reinfection while the case at 197 has a more likely chance to be reinfection. However confirmed reinfection occurring after 48, 51, 59, 63 days respectively of initial infection, has been reported.^{12-14,16} Despite the timeline observed in this study, the previous opinions suggesting that reinfections were unlikely below 90 days due to projected immunity from primary infection is no longer relevant. This will make it more likely for probable reinfections to occur less than 90 days after the first disease to be evaluated.

The evidence of a negative test after a disease episode before repositivity or reinfection is one of the criteria for reinfection with SARS-CoV-2. The new WHO discharge criteria which make the use of a negative test unnecessary, might therefore make it challenging to diagnose reinfections if the confirmation of a negative test post-infection remains a criterion for this assessment.³² In this case, one of the patients did not get a repeat negative test after the first episode. This made it difficult to consider her as probable reinfection, however, the revalidation of the second positive test made it evident that reinfection was excluded. With more patients

being discharged without repeat negative tests, diagnostic algorithms and case definitions for SARS-CoV-2 reinfections should therefore be modified per the current discharge requirements.

The use of algorithms and definitions for the diagnosis of reinfections have been found useful in establishing the probability or otherwise of reinfection in this survey.^{6,7} It is suggested that such criteria as the ECDC and other criteria be revised to improve the diagnosis of probable reinfections from SARS-CoV-2 as shown in this survey.⁶

The results of this survey aimed at identifying probable reinfections in a cohort of SARS-CoV-2 positive patients at a tertiary hospital shows the importance of surveillance and the challenges with access to confirmatory tests for reinfection. The requirement for routine genome sequencing and viral cultures which may not be routine services may also contribute to making diagnosis challenging. It is anticipated that such challenges will be amplified in areas of low resource where issues like logistic in storage and transportation of samples from primary sites to reference labs for genome sequencing and culture prevail.^{6,7,19}

It is therefore advocated that access to routine genetic phenotype and sequencing should be increased in Nigeria, for all cases of presumed or probable SARS-CoV-2 reinfection to establish the real burden of the problem. In the absence of deliberate surveillance as demonstrated in this survey, cases of reinfections will be missed, as patients may not appreciate the significance and are unlikely to report these occurrences. Surveillance for reinfections should therefore be implemented to identify the pattern and trend of reinfections among various populations groups. The implementation of prospective surveillance for reinfections aimed at improving the understanding of predispositions, presentation and patterns of reinfections in Nigeria is also advocated.

This study outcomes also demonstrates how the impact of enhanced knowledge on the subject has changed the perception of SARS-CoV-2 reinfections from a seeming impracticality to realism.

CONCLUSION

The reporting of SARS-COV-2 reinfections globally and the identification of probable reinfection from this survey of a tertiary hospital in Nigeria indicate that reinfections are a global phenomenon and may be more prevalent than documented. The limitations in the technological requirements for confirmation in resource-challenged settings as well as the absence of deliberate surveillance may also be contributory factors. The impact of reinfections on the pandemic control remains uncertain, especially with evidence of reinfections occurring after less than 60 days of first disease and anecdotal reports of 2 or more episodes occurring in patients. Patients with

prior SARS-CoV-2 infection should continue to ensure adherence to IPC advisory as natural immunity is short-lived and reinfection may still cause severe disease. With the onset of vaccinations aimed at preventing SARS-CoV-2 infections, it is advocated that surveillance programs for reinfections should be implemented post vaccinations to improve understanding of immune responses of natural infections and vaccine-derived immunity of SARS-CoV-2 infection.

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