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RESEARCH ARTICLE

Specific Mutations Identified in Patients Vaccinated and Infected with COVID-19 in Senegal

Abdou Padane[1,*], Cyrille Kouligueul Diedhiou[1], Marième Sarr[1], Seyni Ndiaye[1], Abdoulaye Souaré[1], Mame Matar Diop[1], Khadim Gueye[2], Marilyne Aza-Gnandji[1], Ndèye Diabou Diagne[1], Nafissatou Leye[1], Ndèye Dieynaba Diouf[1], Mame Khardiata Ndiaye[1], Ndèye Astou Dabo[1], Ndèye Khady Sow[1], Djibril Wade[1], Souadou Ndoye[1], Abdoul Aziz Diallo[1], Anna Julienne Selbé Ndiaye[1], Yacine Amet Dia[1], Gora Lo[1], Abdoulaye Samba Diallo[1], Ousmane Diop[1], Aminata Mboup[1], Ambroise Ahouidi[1], Papa Alassane Diaw[1], Badara Cissé[1], Moustapha Mbow[1], Makhtar Camara[1], Ndéye Coumba Touré Kane[1], Souleymane Mboup[1]

[1]Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formation (IRESSEF), Dakar, Sénégal.
[2]European Bioinformatics Institute (EMBL-EBI).

Corresponding Author: abdou.padane@iressef.org

ABSTRACT

Background: Vaccination against SARS-CoV-2 is currently the best preventive measure to control the COVID-19 pandemic. However, in some cases, it appeared that despite the vaccination, some people were reinfected.

Aim: The objective of this study is to monitor preliminary data of COVID-19 reinfection cases in vaccinated individuals in Senegal.

Methods: In this study, we used the Oxford Nanopore MinION portable sequencer as detailed in the ARTIC network to test SARS-CoV-2 positive samples from reinfected patients. A total of 71 subjects were monitored with 37 vaccinated patients and 34 non-vaccinated and samples were sequenced in genomic platform at IRESSEF.

Results: We noted the presence of three major lineages B.1.617.2, AY4 and AY34 in vaccinated people. In addition, the mutation W152R and two other mutations never described (T1136S and V1137L) were found in tested genomic sequences.

Conclusion: These results will contribute to monitor future epidemics and to control the effectiveness of the vaccination against COVID-19 especially the Variant of Concern and allow us to improve surveillance for COVID-19 pandemic.

Keywords: COVID-19; SARS-CoV-2; Variant of Concern; Genome; Vaccination; Senegal.

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused more than 270 million new cases since the start of the epidemic [1]. Africa remains the least affected continent but has not escaped the emergence of new variants of SARS-CoV-2 such as the beta variant (March 2020) and the Omicron variant (November 2021), both detected for the first time in South Africa [2,3]. In Senegal, the IRESSEF laboratory was the first to report a case of COVID-19 with the British variant (B.1.1.7)[4].

The Variants Of Concern (VOC) of SARS-CoV-2, Alpha (B.1.1.7), Beta (B.1.351), Gamma (B.1.1.28.1) and Delta (B.1.617.2) have emerged from various countries worldwide (CDC, 2020) and pose a challenge of controlling the COVID-19 pandemic. The Delta variant, first identified in India, is more infectious than the other variants[5]. Furthermore, the Delta variant was associated with SARS-CoV-2 outbreaks and breakthrough infections in vaccinated individuals [6,7]. Previous studies have also reported breakthrough infections following vaccination in India[8,9]. These infectious could be due to emergence of newer mutant strains capable of escaping the host immune response[10]. Indeed, this delta variant is characterized by the presence of a double mutations including one E484K which facilitates immune escape and the other L452R which improves its affinity with human cells[11].

The unevenness of sequencing capacity and the need for decentralization to African regions became clear during the early stages of the pandemic. Research institutes were upgraded to meet the challenge of genomic characterization of SARS-CoV-2 and, by extrapolation, other pathogens. It is in this context that the Institute of Health Research, Epidemiological Surveillance and Training (IRESSEF), located in Dakar, Senegal, has taken up this challenge. The COVID-19 pandemic has shown the limitations that most African countries have in conducting genomic surveillance through sequencing of the virus.

At IRESSEF (Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation), we have successfully implemented a genomic laboratory facility that has performed in real time. This enabled us to identify for the first time the circulation of the Alpha and Omicron variants in Senegal[12,13]. The outcomes of this genomic surveillance contributed to the detection of variants of concern in each pandemic wave as well as other sub-lineages and viral recombinant forms[14]. We then described the phylodynamic evolution of SARS-CoV-2 during COVID-19 in Senegal[15].

To control SARS-CoV-2 infections, much effort has been made with the approval and development of vaccines and antibody therapies. New vaccine techniques are directed against the viral spike protein, but the emergence of viral variants, particularly those in the S gene, threatens their lasting effectiveness [8,16]. The implementation of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a major asset in slowing down the coronavirus disease 2019 (COVID-19) pandemic[17]. Senegalese government had as a main objective during COVID-19 pandemic to obtain 6 million doses of vaccine by the end of 2021[18].

In October 2021, Senegal received 298 700 doses of the AstraZeneca vaccine from India, as well as 324 000 doses of the AstraZeneca vaccine and 200 000 doses of the Sinopharm vaccine through the COVAX initiative[18,19]. These were the three main vaccines used in our country to prevent COVID-19 disease. Senegal began its vaccination campaign to reduce mortality by initially targeting healthcare workers, vulnerable people such as aged and people living individuals at high risk of hospitalization having comorbidities such as heart disease, diabetes, tuberculosis, obesity (Ministry of health in Senegal) [18].

Identifying these optimal vaccination strategies could help policymakers make better decisions for disease control. However, the acceptability of this vaccination posed a real problem due to a lack of awareness and fake news[18].

The new surge of cases in several highly vaccinated countries highlights the need to monitor vaccinated individuals to better deal with the emergence of variants. Thus, we assess in this study the different SARS-CoV-2 variants in fully vaccinated individuals in Senegal. At the same time, an evaluation of mutations involved in the variants is carried out.

Material and methods

STUDY SUBJECT

From July to October 2021, 524 control and vaccinated subjects have been enrolled. For vaccinated subjects, nasopharyngeal and/or oropharyngeal specimen have been collected at day 0, 14 or 28 after the first and second vaccination as well as four and six months after vaccination.

GENOME SEQUENCING

RNA extraction

The oropharyngeal and/or nasopharyngeal samples were first inactivated in a water bath at 60° C for 30 min and then aliquoted in 2 mL vial. RNA was extracted and eluted in 50µL using Kingfisher platform according to the manufacturer's guidelines (www.thermofisher.com: SARS-CoV-2 support and solutions King Fisher instruments and MagMAX isolation kit).

Reverse transcriptase-polymerase chain reaction

The RNA extracts are stored at 4 ° C during the preparation of the Master Mix Allplex™ 2019-nCoV assay from Seegene Inc. were used according to the manufacturer protocol to perform RT-PCR. Briefly, for one reaction, 5µl of 2019-nCoV MOM, 5µl of buffer 5X, 5µl of RNase-free water, 1µl of internal control (IC) and 2µl of enzyme were used. In each well, 18µl of Master Mix were distributed and either 8µl of sample added, 8µl of positive control or 8µl of RNase-free water for negative control. Plates were then spun down at 2500 rpm for 5s and analysed on a CFX96 Touch Real-Time PCR from BioRad, Reverse Transcription reaction using the following setting: 1 cycle: 50°C/20 min – 95°C/15 min, PCR reaction 45 cycles: 94°C/15 sec – 60°C/30 sec– 72° C/15 sec. Fluorescence was measured at 60° C and 72° C using channels FAM (E gene), HEX (IC), Cal Red 610 (RdRP) and Quasar 670 (N gene). Results were compiled and analysed using 2019-nCoV viewer from Seegene Inc. according to the manufacturer's instructions (Seegene. AllplexTM 2019-nCoV Assay) (Cat no. RP10250X / RP10252W).

Libraries preparation

ARTIC protocol from Oxford nanopore was used. Reverse transcription of ARN samples were performed using LunaScript RT SuperMix kit (New England Biolabs, Ipswich, MA, USA). DNA obtained was then amplified by tiled PCR using separate primer pools which were combined, purified, and quantified. Library preparation was done using SQK-LSK-109 and native barcoding (EXP-NBD104, EXP-NBD114 and EXP-NBD196) kits. DNA ends were prepared for adaptor attachment. First ligation native barcodes were supplied before ligation sequencing adapters. Flow cell was priming and DNA library loading into the flow cell.

DATA ANALYSIS

ARTIC analysis workflow [20] was used to assemble the genome. After assembly, sequencing quality control was done. The clade classification and the lineage of each sequence were determined using the Nexclade website [21] and the Pangolin website [22] respectively. Mutations analysis were done and confirmed using GISAID - CoVsurverplatform [23]and check on CoV-GLUE-Viz Mutations[24].

Results

From those, 71 SARS-CoV-2-positive nasal sampling have been found including 37 vaccinated and 34 non-vaccinated subjects who have been screened for SARS-CoV-2 variants. Among vaccinated and positive volunteers, 11 took the Sinopharm, 13 the Astrazeneca Vaccine, 6 for Pfizer and 7 the Moderna. All positive individuals have been recruited either at the 4th or 6th months post vaccination (**Table 1**).

Table 1. Distribution of study population regarding vaccine type in vaccinated group

Vaccine type	**Number of cases**
Sinopharm (SINOVAC®).	11
Astrazeneca (Vaxzevria®),	13
Pfizer-BioNTech (COMIRNATY®)	6
Moderna (Spikevax®),	7
Total	**37**

We sequenced a total of 71 vaccinated and unvaccinated subjects using the ONT platform from 524 enrolled subjects. Bioinformatic Analysis allowed us to identify lineages and sublineages distributed according to the vaccinated group **(Figure 1)**.

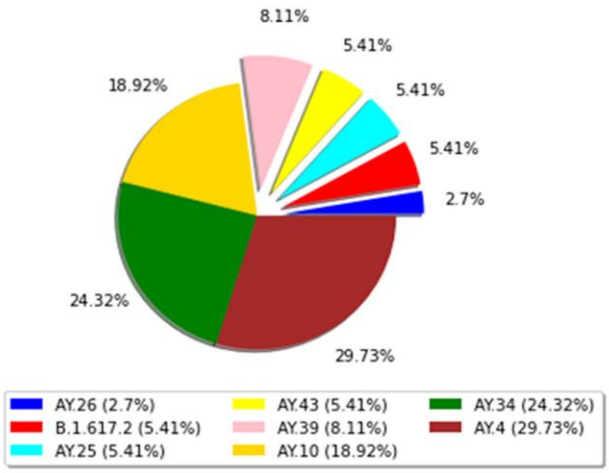

Figure 1. Distribution of Lineages and sublineages identified in the vaccinated group.

People vaccinated with Astrazeneca (Vaxzevria®): In the 13 people vaccinated with Astrazeneca (Vaxzevria®), the following lineages were detected: AY.4 with 53.8% (7/13), AY.34 with 38.4% (5/13) and B.1.617.2 with 7. 6% (1/13).

People vaccinated with Moderna (Spikevax®): In the 7 vaccinated with Moderna (Spikevax®), the lineages AY.34 28.5% (2/7), AY.43 14.2% (1/7), AY.39 14.2% (1/7), AY.25 14.2% (1/7), B.1.617.2 14.29% (1/7) and AY.10 14.2% (1/7) were identified.

People vaccinated with Pfizer-BioNTech (COMIRNATY®): In the 10 patients who have taken the Pfizer-BioNTech (COMIRNATY®), there is a high prevalence of the AY.10 lineage (50% (3/6)) followed by the AY.39 lineage with 33.3% (2/6) and AY .34 with 16.67%. (1/6).

People vaccinated with Sinopharm (SINOVAC®): The most common lineage found were AY.4 with 36.3% (4/11) and AY.10 with 27.2% (3/11) while the AY.34 lineages, AY.43, AY.26 and AY.25 were detected in an equal proportion of 9.1% (1/11). The study of the distribution of mutations according to the patient's status shows two groups. The first group, made up of vaccinated patients with a predominance of the AY.4 and AY.34. The latter is identified in most vaccinated subjects, whatever the vaccine administered. As well as the AY.4 sublineage which is identified in patients who have received the various types of vaccine except for the Moderna vaccine **(Figure 2).**

There was no difference relating to the other sublineages between the vaccinated and the unvaccinated **(Table 2).**

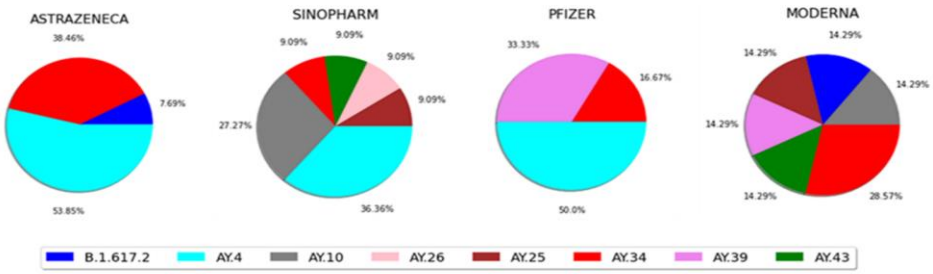

Figure 2. Distribution of Lineages and sublineages identified according to the vaccine used.

Table 2. Distribution of mutations in variants according to vaccinated and unvaccinated.

Mutation	Unvaccinated						vaccinated							
	B.1.617.2	AY.3	AY.4	AY.10	AY.34	AY.37	B.1.617.2	AY.4	AY.10	AY.25	AY.26	AY.34	AY.39	AY.43
A222V	+	+	-	+	-	-	-	-	-	-	-	-	-	-
A262S	+	-	-	-	+	-	-	-	-	-	-	-	-	-
D253G	+	-	-	-	-	-	-	-	-	-	-	-	-	-
D574Y	-	-	-	-	-	-	-	-	-	-	-	-	-	+
D614G	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D950N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E1262G	-	-	+	-	-	-	-	-	-	-	-	-	-	-
E156G	+	+	+	+	-	-	-	-	-	-	-	-	-	-
F157del	+	+	+	+	-	-	-	-	-	-	-	-	-	-
G1219C	-	-	-	-	-	-	-	-	-	-	-	-	-	+
G142D	+	+	-	-	-	-	-	-	-	-	-	-	-	-
L18F	-	-	-	-	-	-	-	-	-	-	-	-	+	-
L1265F	+	-	+	-	+	-	-	+	-	-	-	+	-	-
L452R	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L5F	-	-	-	-	-	-	-	+	-	-	-	-	-	-
N17K	-	-	-	-	-	-	-	-	-	-	-	-	+	-
P499R	+	-	-	-	-	-	-	-	-	-	-	-	+	-
P681R	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Q677H	+	-	+	-	+	-		+				+		
R158del	+	+	+	+	-	-								
R346G	-	-	-	-	-	-						+		
S884F	-	-	-	-	-	-				+				
S477I	+	-	-	-	-	-								
T1136S	-	-	-	-	+	-								
T19R	+	+	+	+	+	+	+	+	+	+		+	+	+
T478K	+	+	+	+	+	+	+	+	+	+	+	+	+	+
T859I	+	-	-	+	-	-	-	-	+	-	-	-	-	-
T95I	+	-	-	-	-	-	-	-	-	-	-	-	-	-
V483A	-	-	-	-	-	-	+	-	-	-	-	-	+	-
V1137L	-	-	-	-	+	-	-	-	-	-	-	-	-	-
V1264L	+	-	-	-	-	+	-	-	-	-	+	-	+	-
Y248H	+	-	-	-	+	-	-	-	-	-	-	-	-	-
W152R	-	-	-	-	-	-	-	+	+	+	-	-	-	+

The second group made up of non-vaccinated patients, have a large dominance of the B.1.617.2 lineage **(Figure 3).**

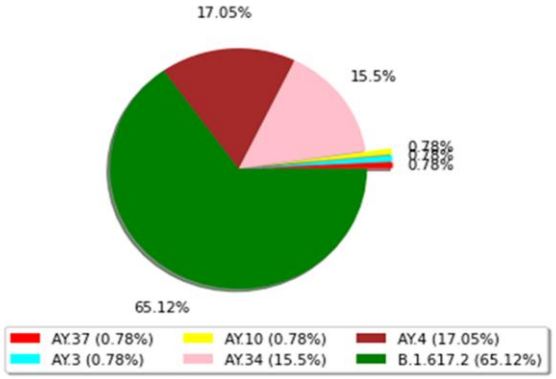

Figure 3. Distribution of Lineages and sublineages identified in the non-vaccinated group.

The sequences analysis revealed the existence of a localized double mutation in the Spike protein, T1136S and V1137L, located at the positions 1136 and 1137, respectively identified. To our knowledge, this is the first time those mutations have been described in the SARS-CoV-2 genome in Senegal and not yet described in the world.

Discussion

In our study, reinfection cases were mainly due to the Delta variant, contrary to some study of [25] which shows 23.7% for Delta compared to 6.6% for all other variants [6]. Indeed, cases of reinfection have been observed in patients vaccinated with Sinopharm, Moderna, Pfizer or Astrazeneca

vaccines. While Tang *et al.*, assess real-world effectiveness of the BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) vaccines against this variant in the population of Qatar [26].

Despite vaccination we observed reinfection in vaccinated patients which is different from what was found in the observational study highlights of Puranik *et al.*, that while both mRNA vaccines from Moderna (mRNA-1273) and Pfizer/BioNTech (BNT162b2) COVID-19 vaccines strongly protect against infection and severe disease progression[27].

In addition, we investigated which sublineages are responsible for this reinfection in patients vaccinated with Sinopharm, moderna, Pfizer or Astrazeneca. Lineages such as AY.4, AY.34 and B.1.617.2 were found in patients vaccinated with Astrazeneca. However, the AY.34 lineage was found in patients vaccinated with Moderna with a proportion of 28.57% of reinfection. In addition, the AY.10 lineage was found in patients vaccinated with Pfizer with a proportion of 50% of reinfection patients followed by the AY.39 lineage. Finally, for Sinopharm, the AY.4 and AY.10 lineage were the most representative. It should be noted that the diversity of lineages identified in individuals vaccinated with Sinopharm was much higher than those in patients using other vaccines.

Specific mutations to the AY.39 lineage of Moderna vaccinated patients such as L18F and N17K were identified.

By comparing B.1.617.2 in vaccinated and unvaccinated patients, we detected a V483A mutation on the spike gene which could be the cause of this resistance. Referring to the CoV-GLUE-Viz Mutations platform containing all mutations available on GISAID, this mutation has never been described in the world to our knowledge. AY.4 in vaccinated and unvaccinated individuals are distinguished by the absence of mutations such as E1156G, E1262, and F157del. However, W152R is present in vaccinated individuals. The latter could also be at the origin of this resistance.

Finally, the global comparison of Delta variants found in vaccinated and non-vaccinated individuals, shows that there are mutations existing only in vaccinated individuals such as D574Y, G1219C, L18F, N17K, R346G, S884F, V843A and W152R. The latter could be the cause of the reinfection which is different from the conclusions of Thangaraj *et al.*,2021 the study findings indicate that the prevalence of B.1.617.2 was not different between the vaccinated and unvaccinated groups[28].

In Senegal, the third wave of the SARS-CoV-2 epidemic was dominated by the Delta variant (Unpublished data). Immunization coverage in Senegal was very low at around 9.78% [29]. The main vaccines used in Senegal are Astrazeneca (Vaxzevria®), Moderna (Spikevax®), Pfizer-BioNTech (COMIRNATY®) and Sinopharm (SINOVAC®). However, reinfection have been noted in vaccinated patients. The Delta variant was the main virus involved in these reinfections.

Exceptionally, we found a double mutation in one patient in the control arm of the unvaccinated patients, mutation T1136S and V1137L, which has never been described. Investigations are still ongoing to determine the origin of this mutation.

Conclusion

The Delta variant discovered in India could give the world's medical authorities a hard time with its batches of mutations, as described in our study shows. This Delta variant continues to progress in Senegal with the appearance of new lineages. All variants found in vaccinated people are Delta lineage and sublineage. This means that cases of Delta infection are becoming increasingly common, despite the use of vaccines in our country. This failure could be due to the appearance of new mutations differentiating the Delta variants identified in vaccinated and non-vaccinated patients. Of these, 39% (13/33) of the mutations on the spike gene are found only in non-vaccinated, 27% (9/33) of the mutations are specific to vaccinated, and 33% (1/33) of the mutations are found in both vaccinated and non-vaccinated. Furthermore, we identified mutations such as W152R, V843A, S884F, R346G and N17K that have never been reported in the spike gene.

Finally, during our study, a particular double mutation was also found at position 1136 of the spike gene (T1136S) and (V1137L) which would deserve further investigation, to understand the underlying mechanisms.

Conflict of Interest:

The authors have declared that no competing interests exist.

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Ethical Approval:

This study was approved by the National Ethics Committee for Health Research of Senegal under the following number: 000159/MSAS/CNERS/Sec, on August 21st, 2020. Free and informed consent is provided by each adult individual who participated in this study.

Author contributions

AP, KG, SM conceived and designed the study. **MS, CKD, SN, NAD, NDD, NKS, MMD** and **AS** performed the experiments. **AP, MM, MS, CKD, SN, KG, YAD** and **DD** recruited study participants and collected data. **AP, MAG, SEN, CKD, MS** and **KG** analyzed and interpreted the data. **SM, PAD, MM, DW, NL, GL** contributed to reagents/materials/analysis tools. **AP, PAD, KG, CKD, AM, AS, GL, NL, AA** and **MC** participated to study design. **AP, SM,** participated to study coordination. **AP, KG, MS, SN and CKD** wrote/drafted the manuscript. **AM, NL, GL, MM, PAD, NCK, SM, AA, GL, MC, MS** and **BC** reviewed critically the manuscript for important intellectual content. **SM, NCK, BC** and **MC** approved the final version to be published. All authors approved the final version of the manuscript.

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