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Detection of the Omicron variant of SARS-CoV-2 by restriction analysis targeting the mutations K417N and N440K of the spike protein.

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Key words: COVID-19; SARS-CoV-2; Omicron Variant of Concern; RFLP; rapid screening.

Abstract. By the end of 2021, the Omicron variant of concern (VOC) emerges in South Africa. This variant caused immediate concern, due to the explosive increase in cases associated with it and the large number of mutations it exhibits. In this study, the restriction sites that allow detecting the mutations K417N and N440K in the Spike gene are described. This analysis allows us to propose a rapid method for the identification of cases infected with the Omicron variant. We show that the proposed methodology can contribute to provide more information on the prevalence and rapid detection of cases of this new VOC.

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Detección de la Variante Ómicron del SARS-CoV-2 por análisis de restricción de dos mutaciones de la proteína de la espiga

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Palabras clave: COVID-19; SARS-CoV-2; Variante Omicron; RFLP; detección rápida.

Resumen. Para finales de 2021 surge la variante de preocupación (VOC por sus siglas en inglés) Ómicron en Sudáfrica. Esta variante causó de forma inmediata preocupación, debido al aumento explosivo de casos asociados a ella y al gran número de mutaciones que exhibe. En este estudio, se describen los sitios de restricción que permiten detectar dos de estas mutaciones en el gen de la espiga, las mutaciones K417N y N440K. Este análisis permite proponer un método rápido para la identificación de casos infectados con la variante Ómicron. Mostramos que la metodología propuesta puede contribuir a proporcionar más información sobre la prevalencia y a detectar rápidamente los casos de esta nueva VOC.

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INTRODUCTION

The COVID-19 pandemic is caused by an emerging coronavirus, SARS-CoV-2, and has caused more than 360 million cases and more than 5 million deaths worldwide. This virus belongs to the family Coronaviridae. The tremendous number of replication events that this virus has experienced, in addition to an elevated frequency of recombination, and the probable action of host deaminases on the viral genome [1], has allowed the emergence of many mutations in the viral genome [2].

Different variants (lineages of viruses sharing particular types of mutations) have emerged since the end of 2020. Some of these variants have been defined as of interest (VOI) or concern (VOC) by WHO, associated with more transmissibility, or partial resistance to protective immunity, among other characteristics. The variants for which at least one of these characteristics has been confirmed, are named VOC [3-7]. There are at present five VOCs: Alpha, which emerged in the UK, Beta in South Africa, Gamma in Brazil, Delta in India, and Omicron in South Africa. Genomic surveillance is recommended for monitoring the introduction of SARS-CoV-2 variants of concern (VOCs) in each country [6, 7].

Since the middle of 2021, the Delta VOC (lineage B.1.617.2) was predominating in many countries and replacing the other circulating variants. However, at the end of November 2021, Omicron VOC (lineage B.1.1.529) was identified in South Africa [8]. This variant was classified as VOC in a record time because of the explosive increase of cases in South Africa, and the great number of mutations exhibited by this new lineage. Since then, up to January 15, 2022, the VOC Omicron has been detected in at least 119 countries (Complete genome sequences submitted in GISAID: https://www.gisaid.org/hcov19-variants/) and is presently replacing Delta worldwide [9].

Omicron VOC exhibits more than 50 mutations in its complete genome, com-

pared to the ancestral Wuhan strain. More than 30 of these mutations are located in the spike protein [8, 10]. Rapid detection of this variant is particularly important, due to the explosive nature of its transmission. The aim of this study was to evaluate a method for rapid detection of this mutation by restriction enzyme analysis, taking advantage of two mutations present in this VOC: K417N and N440K.

MATERIALS AND METHODS

Sequences available at GISAID on January 15, 2022, were analyzed for the presence of two mutations of the Omicron VOC: K417N and N440K. at https://www.gisaid.org. The number of sequences belonging to the Delta VOC among the ones harboring this mutation was also estimated.

This study was approved by the Bioethical Committee of IVIC. A restriction enzyme analysis was developed to detect the presence of two mutations: K417N and N440K. RNA from clinical samples positive by qRT-PCR (classified upon sequencing as Delta or Omicron) were amplified with primers 75L (AGAGTCCAACCAACAGAATCTATTGT) and 76.8R (GTTGCTGGTGCATGTAGAAGTTC) to generate an amplicon of 614 nt, with the PCR conditions previously described [11], with denaturation times of 94°C/30 sec. Six μL of the amplicon were digested with one unit of HindIII or SspI for one hour at 37°C and then loaded in a 3% agarose gel electrophoresis for band visualization with Ethidium bromide. Restriction results were compared with the sequence obtained by sending PCR purified fragments to the Macrogen Sequencing Service (Macrogen, Korea).

RESULTS

Sequences available at GISAID, belonging to the Delta or Omicron variant lineages, were analyzed for the presence of K417N and N440K. A total of 7,136,478 were available at GISAID on January 15, 2022. Only sequences of the complete genome and with high coverage were analyzed. As can be seen in Table 1, while 73% of the sequences available for the Delta variant meet both criteria, only 2% of the Omicron VOC meet them. The presence of K417N or N440K was near 90% in the Omicron VOC lineages, compared to less than 1% in the Delta VOC ones. According to this prevalence data, the detection of one of these two mutations could result in an assay with 94.4% sensitivity and 99.8% specificity.

The analysis of the restriction sites presents in the 614 nt amplicon of the spike gene

Table 1
Number of sequences available at GISAID of Delta or Omicron VOC harboring the mutations K417N and N440K.

Number of sequences (%)*	Delta (B.1.617.2)	Omicron (B.1.1529)
Total sequences	4,061,464	364,148
Sequences of complete genome with high coverage (SCH)	2,954,586 (73%)	5,995 (2%)
SCH with K417N	4,831 (0.2%)	5,415 (90%)
SCH with N440K	99 (0.002%)	5,263 (88%)
SCH with both mutations	0**	5,016 (84%)
SCH with any of the mutations	5,830 (0.2%)	5,662 (94.4%)

*Sequences available at GISAID in January 15, 2022. **12 sequences were available with both mutations among the 4,061,464 total sequences of the Delta variant.

of SARS-CoV-2 showed the presence of a restriction site in the nucleotides corresponding to the K417N and N440K mutations. Fig. 1 shows the expected restriction pattern of samples and isolates harboring mutations K417N and N440K, by using two restriction enzymes: SspI for K417N and HindIII for N440K: the SspI enzyme has an additional restriction site in the respective mutated sample and HindIII an unique restriction site in the mutation N440K. Fig. 1C shows the digestion of the PCR-amplified product with the two enzymes of two samples with the two mutations (Omicron variant) and two samples without the mutations (Gamma or Delta variant). A total of 28 samples were analyzed for their restriction pattern with these two enzymes, and compared for the presence or not of the mutations K417N and N440K in their sequence. A 100% concordance was observed in the detection of the two mutations between the two methods (Table 2). In addition, the sequence of the complete genome was available for three of the samples analyzed, and again a perfect concordance was found with the variant assigned by restriction analysis of the sequencing of the small genomic fragment: two Omicron VCs (GISAID accession numbers EPI_ISL_8063574 and EPI_ISL_8063663) and one Delta VOC (EPI_ISL_8804532). All the Omicron VOCs analyzed in this study harbored both mutations K417N and N440K.

DISCUSSION

For evaluating the projected sensitivity of restriction analysis using these two mutations as an indicator of Omicron presence, sequences available at GISAID were evaluated for the presence of the mutations K417N and N440K. The prevalence between Omicron and Delta VOCs was compared, since the Delta VOC was circulating and predominating in almost every part of the world just before the Omicron VOC rise in cases.

In addition to the huge number of mutations and rise in cases in each country, where this variant began to circulate, another peculiarity of the Omicron VOC is the variability in the number of mutations displayed by each isolate, that is, not all the isolates harbor all the mutations described for this variant. Indeed, the authors found a prevalence of less than 50% for the two mutations analyzed in this study, although they also recognized that the prevalence reported in their study might be higher for some of these mutations [8]. The rush in submitting sequences for the Omicron variant, shown by the huge predominance of complete genome sequences with low coverage (98%, compared to only 27% for Delta VOC, see Table 1), is an important factor that may hamper the real prevalence of each mutation in the Omicron VOC genomes.

According to the analysis performed on the sequences submitted to GISAID, the predicted sensitivity of the proposed restriction analysis, based on the detection of at least one of the K417N or N440K mutations, should result in a test with at least 94% sensitivity. Indeed, the sensitivity could be even higher, considering the possibility that some of the Omicron VOC sequences deposited in GISAID may harbor non-resolved nucleotides in the sites of these two mutations. In addition to few Delta VOCs, the Beta VOC harbors the mutation K417N. Thus, the detection of this mutation might not guarantee the presence of the Omicron VOC. However, the frequency of Beta VOC has been very low in the last months. A search in GiSAID resulted in only six sequences of Beta VOC from December 2021 up to January 2022.

A perfect correlation was found in this study between the restriction and sequence analysis. All the sequences analyzed harbored the two mutations, in agreement with the high prevalence of both mutations found in the sequence analyzed in this study, and providing a predictive value of almost 100% of Omicron identification. This method indeed allowed us to detect the first Omicron cases in Venezuela (Jaspe, RC, and

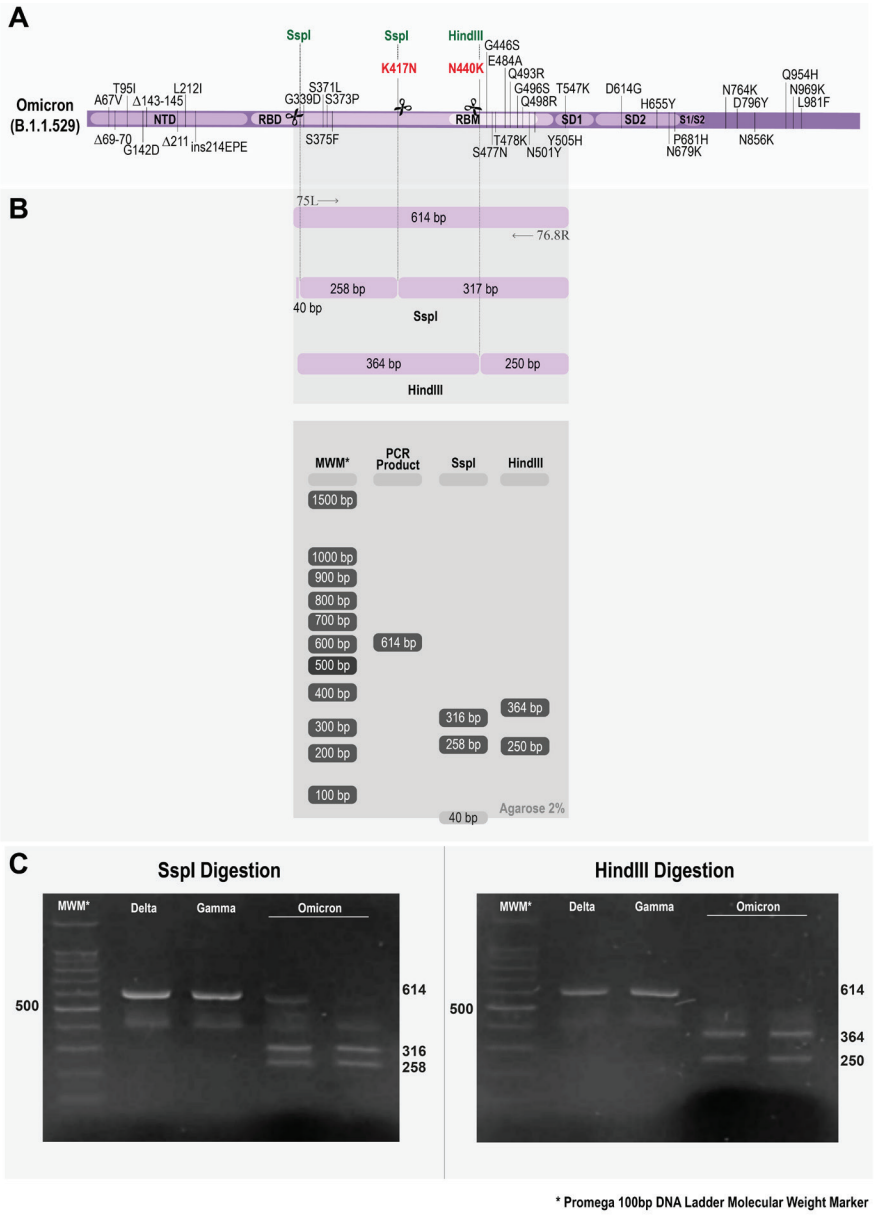

Fig. 1. Restriction analysis of amplicons with K417N and N440K mutations. A. Sequence of the amplified product showing the restriction sites which discriminate Wild-type (WT) or mutant (K417N and N440K) viruses. The use of these two enzymes generates a restriction pattern characteristic for each situation (WT, K417N, and N440K). The restriction sites are underlined. The numbers in the alignment indicate the bp position in the PCR-amplified product. Nucleotides 79-81 code for the amino acid K417 (CTG) or N417 (CGG). Nucleotides 79-81 code for the amino acid N440 (CTG) or K440 (CGG). B. Expected digestion pattern with HindIII or SspI enzymes. With HindIII digestion, the WT amplicon generates an undigested product of 614 bp, while N440K mutated amplicon generates 2 bands: one of 384 pb and one of 250 bp. With SspI digestion, WT amplicon generates two bands: one of 575 pb and one of 40 bp that is not seen in the gel, while K417N mutated amplicon generates three bands of 317, 258, and 40 bp that is not seen in the gel. C. Agarose gel electrophoresis of PCR-amplified products digested with HindIII or SspI. The PCR-amplified products digested with the enzymes were run with molecular weight markers (100bp DNA Ladder Molecular Weight Marker, Promega): smaller bands are signaled (100, 200, and 300 bp).

Table 2
Concordance between restriction enzyme analysis and sequencing results.

Sequence analysis Restriction analysis	K417 and N440 (Delta)	N417 and K440 (Omicron)	Concordance
K417 and N440	15	0	100%
N417 and K440	0	13	

A total of 28 samples were analyzed: 15 Delta VOC (lineage B.1.617.2), without any of the two mutations, and 13 Omicron VOC (lineage B.1.1.529).

Pujol, FH, personal communication). Our group has already developed several restriction analyses for the detection of key mutations present in some VOCs, like E484K and L452R [12, 13]. The method proposed in this study, together with the previously developed for other mutations, has been very useful in our hand for rapid detection of the variant in particular cases. It is important to note that complete genome sequencing is necessary for confirmation of the variant assignment, but once Omicron VOC is expanding in a particular region, the detection of these mutations is highly predictive of its presence.

Due to its explosive reproductive rate, rapid methods for detection of the Omicron VOC are warranted. In our hands, the detection of two of its mutations by restriction analysis was very useful for the rapid detection of the Omicron VOC.

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Declaration of conflict of interest

The authors declare no conflict of interest.

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Authorship contribution

- RCJ and JLZ contributed equally to this study.
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- All the authors read and approved the final version of the manuscript.

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