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SARS-CoV-2 Genomic Surveillance in Nayarit, Mexico (Summer-Winter 2021-2022)

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RESUMEN

Vigilancia genómica del SARS-CoV-2 en Nayarit, México (verano-invierno 2021-2022)

Introducción. El COVID-19, cuyo agente etiológico es el SARS-CoV-2, un virus de ARN, se caracteriza por una elevada tasa de mutación. Por lo tanto, a mayor número de sujetos infectados existe, mayor probabilidad de que el virus sufra cambios que le confieran ventajas evolutivas (evasión de la respuesta inmunitaria, aumento de la virulencia y reducción de la eficacia de la vacunación). Los esfuerzos por adquirir inmunidad de rebaño mediante la vacunación pueden verse comprometidos en los países en vías de desarrollo, donde el proceso de vacunación es lento y poco equitativo. Esto puede dar lugar a nuevos brotes de variantes con mayor capacidad de transmisión.

Objetivo. Vigilar las variantes circulantes en la población Nayarita.

Métodos. En este sentido, en Tepic, Nayarit, México, se secuenciaron 100 genomas virales de pacientes positivos durante el inicio y final de la tercera (4 de agosto al 3 de septiembre de 2021) y cuarta (3 de enero, al 2 de febrero de 2022) olas de COVID-19.

Resultados. El análisis de secuencias reveló la presencia de diversas variantes; alfa (B.1.1.7), gamma (P.1), variante local (B.1.1.519), mu (B.1.621), delta (B.1.617.2), y sus subtipos (AY.3, AY.4, AY.10, AY.11, AY.20, AY.23.1) durante la tercera ola. Posteriormente, durante la cuarta ola, se siguieron detectando subtipos delta (AY.26 y AY.113), así como ómicron (B.1.1.529) y los subtipos de ómicron (B.A.1 y BA.1.1).

Conclusiones. Los datos obtenidos revelaron un desplazamiento progresivo de las variantes dominantes, delta, y subtipos en la tercera ola y ómicron y sus subtipos en la cuarta ola.

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ABSTRACT

Background. COVID-19, whose etiologic agent is SARS-CoV-2, an RNA virus, is characterized by a high mutation rate. Therefore, while more subjects are infected, greater probability that the virus will potentially undergo changes that confer evolutionary advantages (immune response evasion, increased virulence, and reduced vaccination efficacy). Efforts to acquire herd immunity through vaccination may be compromised in low- and middle-income countries, where the vaccination process is slow and inequitable. This may lead to new variant outbreaks with greater transmission capacity. Therefore, it is important to surveillance the circulating variants in the populations.

Methods. In this sense, in Tepic, Nayarit, Mexico, 100 viral genomes of positive patients were sequenced during the beginning and end of the third (August 4th to September 3rd, 2021) and fourth (January 3rd, to February 2nd, 2022) COVID-19 waves.

Results. Sequence analysis revealed the presence of several variants; alpha (B.1.1.7), gamma (P.1), local variant (B.1.1.519), mu (B.1.621), delta (B.1.617.2), and its subtypes (AY.3, AY.4, AY.10, AY.11, AY.20, and AY.23.1) during the third wave. Later, during the fourth wave, delta subtypes were still detected (AY.26 and AY.113), as well as omicron (B.1.1.529) and omicron subtypes (B.A.1 and BA.1.1).

Conclusion. Obtained data revealed a progressive shift of the dominant variants, delta, and subtypes in the third wave and omicron and subtypes in the fourth wave.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is the etiologic agent causing the 2019 Coronavirus Disease (COVID-19). Since the SARS-CoV-2 outbreak in Wuhan, China, more than 690 million cases and more than 6.9 million deaths have been reported worldwide. Mexico has a balance of 7.63 million confirmed positive cases and a mortality of 334,000 patients (1).

SARS-CoV-2 is an enveloped RNA virus that undergoes continued genetic material mutations (2, 3). Without underestimating the biological essence of the virus, viral load, and mutations (variants) are highly pertinent, so there must be surveillance on these parameters. Viral load is essential to understanding viral dynamics, potentially viral load values are higher in patients with worse disease prognosis (4).

Since this virus emerged several lineages of SARS-CoV-2 with an increased ability to affect the population have been identified (5). The World Health Organization (WHO) and experts proposed a classification system for these variants based on their potential public health risks. This system has three main groups: variants of interest (VOI), variants of concern (VOC), and variants under monitoring (VUM). Within these designation criteria, the SARS-CoV-2 variants classified as VOI present changes in the genome that have been shown to affect the viral biological properties, such as transmissibility, disease severity, and ability to evade the immune system. As it has been verified, they give rise to a significant transmission in the community environment causing episodes (epidemiological waves) with an increasing relative prevalence or other characteristics that indicate that they may involve a new risk for global public health (6).

Until January 2023, there are no VOI in circulation in Mexico. VOC presents increased transmissibility, virulence, and decreased efficacy of social measures, vaccines, and treatments. A VUM involves any that has changes in the genome that are suspected to affect viral traits and seem to indicate that the variant may pose risks in the future, despite the lack of evidence. Finally, upon December 2022, no VUMs are in circulation, and all have been classified as Formerly Monitored Variants (FMV), which have been reclassified based on at least one of the following criteria: [1] the variant is not in circulation at levels of global public health significance, [2] the variant has been circulating for a long time without any epidemiological impact, or [3] scientific evidence suggests that the variant is not associated with any concerning properties. The changes that it may cause in viral phenotype or epidemiological characteristics, and it is necessary to maintain monitoring and continue studying it until more information is available (Figures 1 and 2) (7).

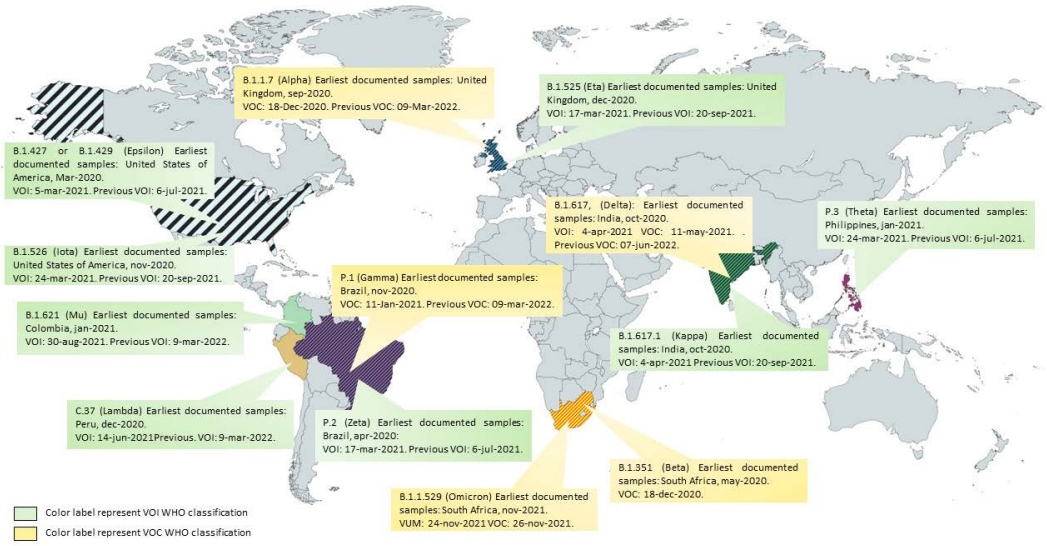

Figure 1. Global distribution of the earliest statement samples as VOC and VOI of SARS-CoV-2 variants from March 2020 until December 2022. The countries with diagonal stripes represent areas with more than one variant documented in this lapse. Adapted from WHO Tracking SARS-CoV-2 variants (8), www.mapchart.net/world.html, accessed on Dec 11th 2022.

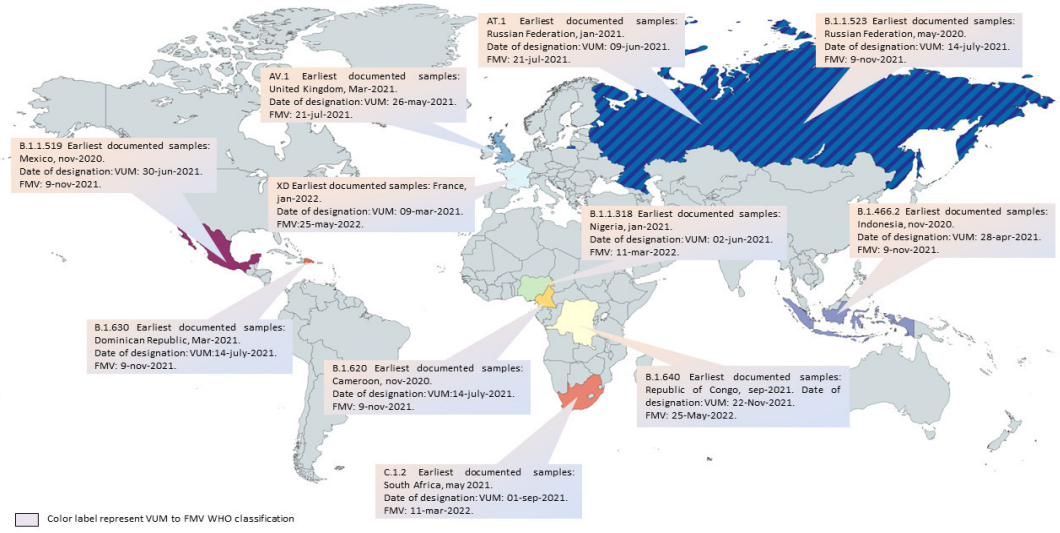

Figure 2. Global distribution of the earliest statement samples as VUM and then now FMV of SARS-CoV-2 variants from March 2020 until December 2022. The countries with diagonal stripes represent areas with more than one variant documented in this lapse. The variants R.1, C.36.3, B.1.214.2, and B.1.619 so far it is not possible to identify their place of origin, therefore they are designated by the WHO as multiple countries. Adapted from WHO Tracking SARS-CoV-2 variants (9-12); www.mapchart.net/world.html accessed on Dec 11th 2022.

At least five VOC (Alpha, Beta, Gamma, Delta, and Omicron), including all sub-variants, affect global public health, as they can increase transmissibility, virulence, and clinical presentation, or decrease vaccination effectiveness. As of March 9th, 2022, the SARS-CoV-2 variants B.1.1.7 (Alpha) (earliest documented samples: United Kingdom-Sep-2020: VOC: Dec 18th-2020), B.1.351 (Beta) (earliest documented samples: South Africa-May-2020: VOC: Dec 18th-2020), and P.1 (Gamma) (earliest

recorded samples: Brazil-Nov-2020: VOC: Jan 11th-2021), B.1.617, (Delta) (earliest documented samples: India-Oct-2020: VOI: Apr 4th-2021 VOC: May 11th-2021), VOCs were categorized as previously circulating VOCs. Only B.1.1.529 (Omicron) (earliest recorded samples: South Africa-Nov-2021: VUM: Nov 24th-2021 VOC: Nov 26th-2021) variants and their subtypes have kept this status (6).

Sequence identification is vital to tracking viral evolution and whether the virus can avoid the effect of the vaccination process (13). In Nayarit, Mexico, the first wave of SARS-CoV-2 occurred between July-August 2020; the second wave in January-February 2021, the third wave in August-September 2021, and the fourth wave in January-February 2022 (14). Mexican health authorities through the Consorcio Mexicano de Vigilancia Genómica (CoViGen-Mex) implemented the surveillance of circulating variants across the National territory in February 2021 (15). Our research group collaborated to analyze samples of positive patients in Tepic City. In this sense, data on circulating variants during the third and fourth COVID-19 waves in this region remain largely unknown. Thus, this study aimed to identify SARS-CoV-2 circulating variants during the third and fourth waves (summer-winter 2021, respectively) in Tepic, Nayarit, a medium-sized city in Mexico.

MATERIAL AND METHODS

Study Design and Participants

Outpatients requesting a qRT-PCR molecular test for SARS-CoV-2 at LANIIA-UAN laboratory (laboratory approved by the Mexican health authorities -number: DGE-DDYR-DSAT05140-2020-), were evaluated. Once SARS-CoV-2 carriers were diagnosed, 100 ambulatory patients were randomly selected for sequencing. This study was conducted during the onset and the end of the third (August-September, 2021) and the fourth COVID-19 wave (January-February, 2022) in Tepic City, Nayarit, Mexico. All patients were duly informed, accepted the use of the information for scientific purposes, and signed an informed consent form before clinical data and sample collection. The local bioethics commission approved this study under registration number CEBN/03/20.

Sample collection

Laboratory personnel were trained to perform the sample collection. Then patients were swabbed (nasopharynx/oropharynx) using two medical grade flocked nylon swabs (iClean®), which were placed on the mucosa while gently swirled for a few seconds, then removed while rotating and placed in 2.5 mL of the sterile viral transport medium (VTM). Sterile VTM (pH 7.10) was prepared according to Girón-Pérez *et al.* (16).

qRT-PCR Procedure

Methods were validated by the Mexican health authorities (Secretaría de Salud de México, and Instituto de Diagnóstico y Referencia Epidemiológicos -InDRE-). All reagents, kits, and procedures were approved by these authorities. Inactivation and total RNA extraction were performed with RNA extraction by QIAmp Viral RNA Mini Kit (Qiagen, Cat No./ID: 1020953 USA, Germantown) with 140 µL of VTM of swabbing samples. The qRT-PCR procedure was performed according to the Berlin protocol with modification (17). One-step qRT-PCR was performed with StarQ One-Step qRT-PCR (Qiagen, Cat No./ID: 210210, USA, Germantown kit). The Viral *E* gene was used for SARS-CoV-2 molecular detection, in all cases, human gene (*RNAseP*) amplification was used as an internal control, and all oligonucleotides and probes sequences are listed in Table 1. One-step qRT-PCR was performed with 5 µL (~70 ng/µL) of extracted RNA in a total 25 µL reaction. All samples were analyzed with ABI Prism 7500 Sequence Detector System (Applied Biosystems) using the following protocol: 50°C for 15 minutes, 95°C for 2 minutes, and then 45 cycles of 95°C for 15 seconds 82°C, and 60°C for 30 seconds. Samples were considered positive if the number of cycles needed for the fluorescent signal to cross the threshold cycle, known as the cycle threshold (Ct) value, was lower than 38.

Table 1. Oligonucleotides sequences used for qRT-PCR detection of SARS-CoV-2 (17).

Molecular target	Primers and probes sequences
E gene (virus)	E_Sarbeco_Forward: ACAGGTACGTTAATAGTTAATAGCGT E_Sarbeco_Reverse: ATATTGCAGCAGTACGCACACA TaqMan probe: FAMCACTAGCCATCCTTACTGCGCTTC G-BBQ
RNAseP gene (Human)	RNAseP Forward: AGATTTGGACCTGCGAGCG RNAseP Reverse: GAGCGGCTGTCTCCACAAGT TaqMan probe: FAMTTCTGAC-CTGAAGGCTCTG CGCG-BHQ1

SARS-CoV-2 sequencing

Positive samples were collected from August 4th to September 3rd, 2021, and January 3rd to February 2nd, 2022, corresponding to the third and fourth COVID-19 waves in Mexico, respectively. Samples positive for SARS-CoV-2 by qRT-PCR (Ct<20), were selected for sequencing at the Microbial Genomics Laboratory, CIAD-Mazatlán, belonging to CoViGen-Mex.

A total of 100 samples, previously cleaned to eliminate adapters, primer sequences, and low-quality bases (<Q30) were analyzed. Reads were further cleaned with fastp (-q 30 -l 20 --low_complexity_filter) and then mapped and indexed to the Wuhan reference sequence (NC_045512.fasta) with bwa-0.7.17-r1188 and samtools 1.10. A consensus sequence for each sample and variations from the reference sequence were obtained with samtools 1.10 e iVar version 1.3.1. All consensus sequences were classified with Pangolin 3.0.3 to obtain the SARS-CoV-2 lineage.

RESULTS

From August 2021 to February 2022, a total of 100 whole SARS-CoV-2 genomes were randomly selected for sequencing and variant identification. The patients were residents of the city of Tepic, Nayarit, Mexico. All patients were exclusively outpatients and diagnosed with a high viral load since threshold cycles lower than 20 were selected for sequencing. Of the selected samples, 57 were men (average age of 39.79 and an age range from 7 months to 73 years) and 43 were women (average age of 38.42 and an age range of 14 to 76 years).

Out of 100 samples in the study area during the third and fourth waves, the circulating variants classified as VOI were as follows: alpha (B.1.1.7) with only 2 samples, gamma (P.1) with 5 samples, delta (B.1.617.2) with 20 samples, and omicron (B.1.1.529) with 18 samples. The only variant previously classified as VOI was mu (B.1.621), and in the VUM category, only the Mexican variant (B.1.1.519) was detected, each with a single sample. Among the most diverse lineages, those from the delta variant were identified, including AY.3 (6 samples), AY.4 (2 samples), AY.10 (5 samples), AY.11 (3 samples), AY.20 (2 samples), AY.23.1 (1 sample), AY.23 (4 samples), and AY.113 (1 sample). For omicron, only two lineages were detected: BA.1 (8 samples) and BA.1.1 (21 samples) (Figure 3).

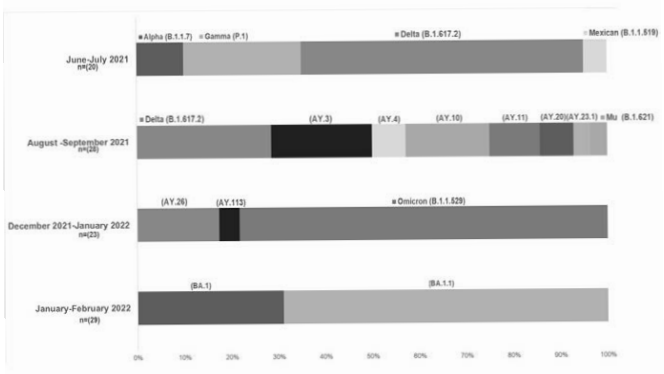

Figure 3. Stacked graphs of the circulating variants during the third and fourth waves in the city of Tepic, Nayarit, Mexico.

DISCUSSION

Many biological, socio-economic, and cultural factors were responsible for the current uncontrolled spread of SARS-CoV-2 throughout the world (18). Factors such as lack of health infrastructure, gaps in vaccine coverage, vaccination hesitancy, inequitable distribution, and mistrust of people regarding the quality, safety, and efficiency of the available

vaccines, as is the case in regions such as low- and middle-income countries, promote those geographic regions present an elevated risk for the emergence of new SARS-CoV-2 variants, thus representing a global public health risk (19-22).

Until December 2022, the dominant variant worldwide is Omicron, including BA.1, BA.2, BA.3, BA.4, BA.5, and descendent lineages. It also includes BA.1/BA.2 circulating recombinant forms such as XE, XBB, and BQ.1(6). Particularly in Mexico, several variants classified as VOI, VOC, or VUM, 16 viral lineages have circulated during the period from March 2020 to February 2021, the most dominant of which were B.1, B.1.1, B.1.1.222, B1.243, B.1.609, and B1.1.519; where the latter lineage was dominant in late 2020 and early 2021 (23).

Detection of multiple variants circulating at the same time in the population has also been reported in other countries (24-26). With the new SARS-CoV-2 variants outbreaks, populations face epidemiological waves, in which the emerging variants generally displace the earlier circulating variants (27). In this research a similar fashion phenomenon occurred; at the beginning of the third wave in Mexico, the alpha (B.1.1.7), gamma (P.1), delta (B.1.617.2), and the Mexican variant (B.1.1.519), were detected. Then, at the end of the third wave, the delta variant and its subvariants predominated (AY.3, AY.4, AY.10, AY.11, AY.20, AY.23.1), although the mu variant was also detected (B.1.621). Later, during the beginning of the fourth wave, delta subvariants were still detected (AY.26 and AY.113), despite the omicron variant having a higher presence (B.1.1.529). Finally, at the end of the fourth wave, only two omicron subvariants were detected (B.A.1 and B.A. 1.1) (Figure 3).

Application of Genomics-based surveillance in time scales by geographic zones allows us to identify circulating and predominant variants to assess the evolution of the SARS-CoV-2 epidemic (28, 29). Additionally, the outbreaks of variants also impact the symptomatology, hence, there are variants of higher risk than others (30). In line with this, the rise in cases of the third and fourth waves can be attributable to the delta and omicron variants, as shown in Figure 3, given that both variants have increased transmissibility, exhibiting a greater capacity to infect cells and evade the immune response in comparison with the native variant (31-35).

Mutations harboring these variants confer greater transmissibility, sometimes increasing severity and immune evasion (neutralizing antibodies and cellular response) compared to the original strain. The current vaccination strategy, which includes different doses, protects against severe diseases. As of the beginning of 2022, most of the 33 vaccines have been approved for use in 197 countries, which contributes to decreasing the spread of the disease (36).

CONCLUSION

VOCs were identified during the third and fourth waves of COVID-19. A transition of the predominant circulating variants throughout the pandemic during the third (August 4th to September 3rd, 2021) and the fourth waves (January 3rd to February 2nd, 2022), was observed. Surveillance is of utmost importance to track new mutations and predominant circulating variants for the implementation of public health policies.

Study limitations: Some study limitations exist. Screening bias exists since specimens were sorted by cycle threshold values (Ct<20). In addition, all study participants were exclusively outpatients, whereas hospitalized patients were not analyzed by our facilities.

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Institutional Review Board Statement: This study was performed following the guidelines stated by the Commission of Nayarit State, Mexico (registry number CEBN/03/20).

Informed Consent Statement: All study participants signed the informed consent before sampling and information data collection, following a protocol approved by the local bioethics commission (registry number CEBN/03/20).

Conflicts of Interest: The authors declare no conflict of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author, Girón-Pérez M.I, upon reasonable request.

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