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Community SARS-CoV-2 Dynamics and Cycle Threshold Use to Enhance Public Health Surveillance in the Dominican Republic.

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Community SARS-CoV-2 Dynamics and Cycle Threshold Use to Enhance Public Health Surveillance in the Dominican Republic.

Abstract

To propose appropriate containment measures and optimize surveillance strategies, it is of utmost importance to understand the dynamics of SARS-CoV-2 transmission within different age groups and its symptomatic and asymptomatic presentations. This study aimed to understand the cycle-threshold (Ct) of SARS-CoV-2 within individuals attending hospital-based facilities in the Dominican Republic. A total of 3,309 saliva samples were analysed from 14 provinces, which yielded a positivity rate of 18.0% (n=596) across 24 sites. Saliva specimens and levels of viral RNA were quantified by RT-qPCR. Overall mean Ct values were 29.3 cycles and significantly correlated with community positivity rate (r=-0.034, p=0.04). There was no significant difference in mean Ct values between studied age groups [F (19, 30) = 0.65, p = 0.5] and no significant correlation between mean Ct by age groups and community positivity. When comparing asymptomatic and symptomatic patients by age groups, the patients between 5 and 17 years old demonstrated a statistically significant mean difference in Ct values with 27.5 and 32.4 cycles, respectively (t (14) = -2.3, p = 0.03). Results identified in this study demonstrate how understanding community viral load is crucial for optimal SARS-CoV-2 surveillance and should be evaluated in the context of transmissibility dynamics.

Keywords: COVID-19, Ct value, Dominican Republic, PCR, RdRp Gene, Viral load, Transmission dynamics.

Resumen

Para proponer medidas de contención adecuadas y optimizar las estrategias de vigilancia, es de suma importancia comprender la dinámica de transmisión del SARS-CoV-2 en los diferentes grupos de edad y sus presentaciones sintomáticas y asintomáticas. Este estudio tuvo como objetivo comprender el ciclo-umbral (Ct) del SARS-CoV-2 en personas que asisten a instalaciones hospitalarias en la República Dominicana. Se analizaron un total de 3.309 muestras de saliva de 14 provincias, lo que arrojó una tasa de positividad del 18,0 % (n=596) en 24 sitios. Las muestras de saliva y los niveles de ARN viral se cuantificaron mediante RT-qPCR. Los valores medios generales de Ct fueron 29,3 ciclos y se correlacionaron significativamente con la tasa de positividad de la comunidad (r=-0,034, p=0,04). No hubo diferencia significativa en los valores medios de Ct entre los grupos de edad estudiados [F (19, 30) = 0,65, p = 0,5] y no hubo una correlación significativa entre el Ct medio por grupos de edad y la positividad de la comunidad. Al comparar pacientes asintomáticos y sintomáticos por grupos de edad, los pacientes entre 5 y 17 años demostraron una diferencia de medias estadísticamente significativa en los valores de Ct con 27,5 y 32,4 ciclos, respectivamente (t (14) = -2,3, p = 0,03). Los resultados identificados en este estudio demuestran cómo la comprensión de la carga viral de la comunidad es crucial para una vigilancia óptima del SARS-CoV-2 y debe evaluarse en el contexto de la dinámica de transmisibilidad.

Resumo

Para propor medidas de contenção adequadas e otimizar estratégias de vigilância, é de extrema importância compreender a dinâmica da transmissão do SARS-CoV-2 em diferentes faixas etárias e suas apresentações sintomáticas e assintomáticas. Este estudo teve como objetivo entender o ciclo-limiar (Ct) do SARS-CoV-2 em indivíduos atendidos em instalações hospitalares na República Dominicana. Um total de 3.309 amostras de saliva foram analisadas de 14 províncias, o que rendeu uma taxa de positividade de 18,0% (n=596) em 24 locais. Amostras de saliva e níveis de RNA viral foram quantificados por RT-qPCR. Os valores médios gerais de Ct foram de 29,3 ciclos e significativamente correlacionados com a taxa de positividade da comunidade (r=-0,034, p=0,04). Não houve diferença significativa nos valores médios de Ct entre as faixas etárias estudadas [F (19, 30) = 0,65, p = 0,5] e não houve correlação significativa entre Ct médio por faixas etárias e positividade da comunidade. Ao comparar pacientes assintomáticos e sintomáticos por faixa etária, os pacientes entre 5 e 17 anos demonstraram diferença média estatisticamente significativa nos valores de Ct com 27,5 e 32,4 ciclos, respectivamente (t (14) = -2,3, p = 0,03). Os resultados identificados neste estudo demonstram como a compreensão da carga viral da comunidade é crucial

para a vigilância ideal de SARS-CoV-2 e deve ser avaliada no contexto da dinâmica de transmissibilidade.

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Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) the microbe responsible for the current coronavirus disease 2019 (COVID-19) pandemic. Although this novel enveloped RNA coronavirus was first identified in Wuhan, China, in December 2019, the first report of a SARS-CoV-2 infection in the Dominican Republic was on the 29th of February 2020. [1, 2] The Dominican Republic put in place a diverse set of interventions to prevent the aggressive spread of this viral agent soon after its first reported case. These interventions were based on strategies that had been proven effective during previous epidemics, included but were not limited to educational campaigns, clinical interventions such as case detection and hospitalization of infected individuals, as well as isolation and quarantine measures. [1,3] Despite herculean efforts to combat its propagation, the virus's spread remains uncontrolled.[4] SARS-CoV-2 demonstrates equal susceptibility for infection regardless of age or gender. The virus can cause disarray of symptom, severity, and transiently among different age groups, particularly among younger populations that tend to be asymptomatic and thus relativity under-diagnosed. [5,6,7] This is notable because younger populations could potentially become a risk factor for the further spread and cryptic transmissibility of the disease within the family members. [5] According to official data in the Dominican Republic, the IQR of infections by SARS-CoV-2 is between 28 to 52 years old. [4] Moreover, to propose appropriate containment measures and optimize surveillance strategies, it is of utmost importance to understand the dynamics of SARS-CoV-2 transmission within different age groups and its symptomatic and asymptomatic presentations. This study aimed to understand the cycle-threshold (Ct) of SARS-CoV-2 within individuals attending hospital-based facilities in the Dominican Republic and use this data to identify critical transmission pockets among the population as an indirect indicator for strengthening future public health policies.

Materials and Methods

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A total of 3,309 saliva samples were analysed from 14 provinces in the Dominican Republic across 24 hospital sites between June and September 2020 (coinciding with the first SARS-CoV-2 wave, and previous to mass vaccination efforts) (Figure 1A). Sample collection occurred in hospital-based facilities across the national territory that the Ministry of Health identified as prioritized facilities for screening and treating SARS-CoV-2 infected individuals and included all hospitalized patients, symptomatic out-clinic patients, and referred known contacts. Demographic forms were collected from individuals from 465 positive samples (14%), including general assessment, suspected case criteria (fever, cough, or previous contact with suspected or confirmed cases), clear signs and symptoms, symptoms severity, and infection outcomes not recorded. For data analysis, the date of symptom onset was recorded as “Asymptomatic”, “Between 1-6 days”, “Between 7-13 days” and “Over 14 days”. To prevent recall bias, participants who couldn’t recall the presence of symptoms were recorded as “Unreported”. Negative cases and incomplete forms or duplicates were excluded in the analysis. Saliva specimens were collected at each of the specified facilities and referred to the Molecular Biology Laboratory at the Institute for Tropical Medicine & Global Health at Universidad Iberoamericana (IMTSAG-UNIBE) within the first 24 hours from the time of collection. All saliva samples were processed by automatic nucleic acid purification using Advansure E3 System (LG chem, Pennsylvania), and levels of viral RNA were determined by a reverse transcriptase quantitative PCR (RT-qPCR) using the PowerChek 2019-nCoV Real-Time PCR Kit (kogenebiotech, Korea). According to the manufacturer's protocol for the AB Fast 7500 Real-time PCR System (Applied Biosystems), under the Rn threshold of 0.2, amplification and replication were performed. The results were considered "Detected" when the cycle threshold (Ct) of the RdRP gene amplification was 37 or less, and "Undetected" when the Ct was greater than 37 or when there were no amplification curves detected. The Ct value from the RT-PCR is inversely proportional to the sample's

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viral load so that Ct value can serve as a proxy for viral load. We analysed the Ct values of all consecutive patients in our laboratory who tested positive for SARS-CoV-2.

Results

The sample yielded a positivity rate of 18% (n=596). Overall mean Ct values were 29.3 cycles and significantly correlated with community positivity rate (r=-0.034, p=0.04). (Figure 1B) Among positive individuals, the mean age was 39.8 years old [SD= 15]. Our samples' primary age distribution consisted of patients aged 35 to 54, who made up 40% (n=188) of the sample population. (Table 1) We found no significant difference in mean Ct values between studied age groups [F (19, 30) = 0.65, p = 0.5] (Table 2) and no significant correlation between mean Ct by age groups and community positivity (Figure 1C). Our Findings indicated no overall difference in mean Ct value when comparing symptomatic and asymptomatic patients, with symptomatic patients having a mean value of 29.4 cycles and asymptomatic patients having a mean value of 29 cycles (t (140) = 0.52, p = .6). In contrast, correlation analysis indicates that mean Ct values in asymptomatic individuals significantly and inversely correlate with community positivity rate (r=-0.43, R^2=0.181, p=0.0002).

Additionally, statistically significant evidence of differences in Ct values was found when comparing time elapsed from the date of onset of symptoms to the RT-qPCR test [F (3, 319) = 6.6, p = 0.0002]. Mean Ct value for patients with over 14 days since onset of symptoms (M=32.9, SD=3.5) was significantly higher when compared with the asymptomatic individuals (M=29, SD=6), the patients that had an onset of symptoms between 1 to 6 days (M=28, SD= 5.4), and those with an onset of symptoms between 7-13 days. (29.2, SD=5.9). No difference was found in mean Ct values between asymptomatic individuals and those with 1 to 13 days since the onset of symptoms. Nor was there a difference found in CT values bet ween patients who survived the infection (M=29.4) and those who succumbed to the disease (M=31) (t (42) = 0.5 p = 0.3).

Discussion

Results from positivity rates were compared with RT-qPCR-Ct during sample analysis, which later was used as a proxy of real-time geographic viral dynamics. Due to disparities in the proportion of symptomatic carriers in different age groups, viral load differences were also compared between symptomatic and asymptomatic patients.[8]

Studies have suggested that differences in symptomatic proportions of SARS-CoV-2 infections by age groups could lead to under detection and underestimating transmissibility in younger populations. [5,6,7] (Table 2) However, as stated above we found no significant difference in mean Ct values between studied age groups and no significant correlation between mean Ct by age groups and community positivity (table 1). These findings indicate that viral loads are comparable between age groups, and that age does not seem to be an independent factor guiding SARS-CoV-2 transmissibility.

Studies like Fajnzylber et al and Chen X et al have found an association between viral load and disease severity in infected populations.[11,12] Whereas, our findings indicate no overall difference in mean Ct value when comparing symptomatic and asymptomatic patients as a whole, with mean Ct values in asymptomatic individuals significantly and inversely correlate with community positivity rate emphasizing the importance of understanding the role of younger, asymptomatic patients within the pandemic, where they can go undetected by surveillance systems but could potentially exhibit higher viral load levels than symptomatic individuals.

Governing bodies should also evaluate the number of days individuals should remain isolated after viral transmission based on the findings that viral load trends decreased to undetected levels only after 14 days of symptom onset, in line with what others have previously found, considering viral molecular profiles when molecular surveillance data is collected [8,14],

Our results found no appreciable difference in mean Ct values between asymptomatic individuals and those with 1 to 13 days since the onset of symptoms, which contribute to the understanding that asymptomatic patients can serve as potential SARS-CoV-2 viral drivers with significant public health implications. In addition, no difference was found in Ct values between patients who survived the infection and those who succumbed to the disease, demonstrating that viral load levels are not necessarily a driver in severity differences.

Results from this study punctuate the need to reframe public health measures and incorporate strategies that include proper infection detection in younger and asymptomatic populations. Surveillance strategies should focus on monitoring a representative sample size of these specific populations to capture the entire caseload and community viral load levels of SARS-CoV-2. Enhancing widespread surveillance strategies can open the possibility of detecting those infected individuals that would have otherwise been unaware of their status and serve as community transmission pockets. This study highlights the use of community viral load as a potential tool in monitoring SARS-CoV-2.

Surveillance systems should consider monitoring the evolution of apparently unaffected asymptomatic individuals, especially children, who play a vital role in viral propagation, as suggested by other studies in the Dominican Republic and other parts of the world. [6,13] To understand the natural transmission dynamics of SARS-CoV-2 in our communities, future studies should focus on establishing the role asymptomatic carriers play in infectious circulation. [9,10]

One of the limitations identified in our study was the absence of information indicating symptoms severity, limiting our understanding of viral load's role with these variables.

Because of this, we were unable to assess the contact transmission role and viral load transmissibility ratios; however, some studies have found that not all detected individuals could transmit the infection, and little data exist of the threshold for transmissibility of SARS-CoV-2 compared with viral load.[14,15,16] To counteract these limitations, our hypothesis was restricted to symptom reports rather than specific symptom severity and assumed all detectable individuals could similarly transmit the infection, as many surveillance systems worldwide suggest. Another limitation this study faced was that at this time in the pandemic, COVID-19 tests were restricted due to logistic and geographical availability, therefore were offered passively, and participants had to attend a hospital to acquire an exam. The working class mainly utilized these services, who were required to show a negative PCR test to reintegrate to work. This limited lower and upper ranges of ages in our sample and gender distribution, considering that asymptomatic children were still not included in surveillance strategies at this point in the Dominican Republic.

We conclude that understanding community viral load is crucial for optimal SARS-CoV-2 surveillance and that the addition of SARS-CoV-2 viral load measurements to the current surveillance system monitoring efforts would enhance surveillance efforts by correlating viral load with the community's positivity rates.

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Conflicts of interest: None declared

Ethical approval: The institutional review board approved this study at Universidad Iberoamericana (UNIBE) #CEI-2020-16, and the National Bioethical Committee (CONABIOS) #020-2021.

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R.P.R. and L.T. conceived of the study. L.T., A.V.D, E.S and R.P.R. designed the analyses. A.V.D., D.H., D.O., J.C., and J.M.C. processed the samples. L.T., A.P., C.S., C.M., L.M., M.D., S.T., J.S.C., P.M.D.C., A.V.D, E.S. and R.P.R. all contributed to data analysis. All authors contributed to the writing of the paper.

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Tables & Figures

Table 1: Descriptive statistics of Positive RT-qPCR for SARS-CoV-2 in the Dominican Republic. (N=465)

Demographic Characteristics	**Mean Ct**	**n (%)**
Sex		
Male	29.2	206 (44.3%)
Female	30.1	259 (55.7%)
Age (yo)		
Mean Age: 39.8		
5-17	31.1	23 (5.0%)
18-34	29.7	174 (37.4%)
35-54	29.7	188 (40.4%)
55<	29.3	78 (16.8%)
Date of Symptom Onset		
Asymptomatic	29.0	85 (18.3%)
1-6 days	28.1	81 (17.4%)
7-13 days	29.2	119 (25.6%)
14 or more days	32.9	38 (8.2%)
Unreported	30.1	142 (30.1%)
Provinces (Positivity Rate)		
Duarte **(34.8)**	34.6	14 (3.0%)
Elias Pina **(14.3)**	31.1	11 (2.4%)
La Altagracia **(10.7)**	30.7	20 (4.3%)
Distrito Nacional **(21.6)**	30.5	52 (11.2%)

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Santiago **(13)**	30.5	16 (3.4%)
Santo Domingo **(12.5)**	30.4	76 (16.3%)
Azua **(16.3)**	30.2	20 (6.2%)
San Cristobal **(24)**	30.0	83 (18.0%)
San Pedro de Macoris **(19.2)**	29.3	32 (6.9%)
Espaillat **(11.7)**	28.8	24 (5.2%)
San Juan de la Maguana **(12.6)**	28.6	30 (6.5%)
La Vega **(42.5)**	28.1	18 (3.9%)
Barahona **(20)**	27.5	52 (11.2%)
Maria Trinidad Sanchez **(30)**	26.4	8 (1.7%)

Table 2: Descriptive statistics of Positive RT-qPCR for SARS-CoV-2 in the Dominican Republic by Date of Symptom Onset. (N=465)

	Date of Symptom Onset n (Mean Ct)				
	Asymptomatic	1-6 days	7-13 days	14 or more days	Unreported
Sex					
Male	41 (29.0)	37 (27.8)	48 (27.6)	18 (33.7)	62 (30.0)
Female	44 (29.0)	44 (28.3)	71 (30.3)	20 (32.1)	80 (31.1)
Age (yo)					
Mean Age: 39.8					
5-17	6 (27.5)	4 (28.3)	5 (34.8)	1 (37.0)	7 (32.3)
18-34	27 (29.2)	38 (27.8)	43 (29.6)	8 (32.3)	58 (31.0)
35-54	37 (29.4)	28 (27.5)	47 (29.2)	19 (32.4)	57 (30.4)
>55	14 (28.0)	10 (29.6)	24 (27.4)	10 (34.0)	20 (29.9)

Figure 1A. Sample flow diagram and analysed samples for SARS-CoV-2 detection between June-September 2020; B. Positivity Rate of SARS-CoV-2 in selected communities in the Dominican Republic; C. Mean Ct value distribution of SARS-CoV-2 individuals in selected communities in the Dominican Republic. Maps were generated under ArcGIS software ver.10.8.1 license