

Online symptoms screening and testing of Covid-19 through RT-LAMP saliva of students and asymptomatic employees in a public school in Brazil.

Silvia Figueiredo Costa (✉ silviacosta@usp.br)

Universidade de São Paulo

Erika Regina Manuli

Universidade de São Paulo

Beatriz Araujo Oliveira

Universidade de São Paulo

Fábio Eudes Leal

Fábio Eudes Leal

Edgar Casado Barreta Souza

Escola Municipal Oscar Niemeyer

Ana Paula Illi

Escola Municipal Oscar Niemeyer

Ana Paula Barreto de Paiva

Universidade de São Paulo

Camila da Silva Fachini

Universidade de São Paulo

Beatriz Aparecida Munhoz Cano

Universidade de São Paulo

Priscila de Lima Barros

Universidade de São Paulo

Ligia Capuani

Departamento de Tecnologia da Informação, Modular Research System Ltda, São Paulo, Brasil.

Helves Humberto Domingues

Departamento de Tecnologia da Informação, Modular Research System Ltda, São Paulo, Brasil.

Maria Rita dos Santos e Passos-Bueno

Universidade de São Paulo

Ester Cerdeira Sabino

Universidade de São Paulo

Short Report

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Abstract

Objective

To evaluate a respiratory symptoms screening system and test asymptomatic individuals (high school students and employees) at a public school in Brazil.

Methods

SARS-CoV-2 reverse transcription loop-mediated isothermal amplification (RT-LAMP) of saliva samples was performed weekly.

Results

A total of 969 samples were tested (mean of 108 tests per week). No asymptomatic participants tested positive for COVID-19, and no cases of COVID-19 transmission occurred at school despite the high transmissibility of the Delta variant, the local predominant variant at the time of the study.

Conclusion

Implementation of an online system of respiratory symptom screening and testing of COVID-19 through saliva in asymptomatic individuals is a feasible, low-cost and practical option to be used, especially in low-income countries.

Introduction

Children will be the last to be vaccinated against COVID-19, as the vaccine was just recently approved for children from 5–11 years old and it is not approved yet for children under 5 years of age. In this scenario, schools can be a site of dissemination of COVID-19, leading to outbreaks and a surge in new variants[1].

Many countries have implemented respiratory symptom screening and contact tracing at schools[2, 3]. In Brazil, more than 180,000 schools were closed, and 47 million students were on distance education (e-learning) during the first year of the COVID-19 pandemic[4].

In 2021, the second year of the pandemic, schools adopted a mixed classroom education model including distance education and in-person classes following the World Health Organization (WHO) recommendations of physical distance, hand hygiene and the use of a mask. However, data regarding respiratory screening systems and contact-tracing strategies in schools in middle-income countries such as Brazil are scarce.

The aim of this study was to evaluate a respiratory symptom screening system and test asymptomatic individuals using reverse transcription loop-mediated isothermal amplification (RT-LAMP) on saliva samples.

Methods

Setting

The COVID-19 symptom surveillance system was adopted at the Oscar Niemeyer Municipal (ONM) public school in São Caetano do Sul (SCS), Brazil[6], which has 428 students in elementary school, 431 in high school and 83 employees. The school provided free three-layer surgical masks for students and employees and N95 masks for employees.

Study design

This was a descriptive observational study conducted over eight weeks, from September 13th to November 17th, 2021.

The study was publicized to students, family members and staff through posters, WhatsApp messages and a video (**video alunos 4_exportar.mp4**). This project was approved by the Ethics Committee, protocol number CAAE 50555921.6.0000.5510; all participants signed a free informed consent form.

The participants were invited to complete a questionnaire via the website or by phone that included sociodemographic data, information on symptom type, and vaccination status. Before going to school, participants answered a respiratory symptoms questionnaire with 5 questions (presence of fever, cough, runny nose, loss of smell)[6].

Participants reporting COVID-19 symptoms (i.e., having at least two of the following symptoms: fever, cough, sore throat, coryza, and anosmia) were evaluated and referred to a primary care clinic[6].

Saliva Collection

Individuals were asked to refrain from eating, drinking or brushing their teeth for at least 30 min before self-collection of 3 mL of saliva in sterile, nuclease-free 15 mL conical tubes. Inactivated saliva samples were mixed with a solution called DGS at a dilution of 1:2 and then incubated at 55°C for 5 minutes[7].

RT-LAMP

Previously published primer sets targeting different regions of the SARS-CoV-2 genome and the human ACTB gene were used[7, 8]. RT-LAMP reactions (12.5 μ L total volume) containing 1x WarmStart® Colorimetric LAMP Master Mix (New England Biolabs, Ipswich, MA, USA; #M1800L) were carried out at 65°C for 30 to 40 min[7].

Results

Among the 431 students enrolled in high school at ONM school, an average of 32,5% of students attended classes daily for five hours in person during the test period. The total number of participants in the study was 200 individuals, 59 employees (28 professors) and 141 students (Fig. 1).

Approximately 47 employees fully completed the initial questionnaire (80%); all were fully vaccinated (at least two weeks of second doses); 25 received Coronavac, 18 Astrazeneca and 4 Pfizer. Among students, 97 completed the initial questionnaire (69%), 87 received the first dose of Pfizer, 4 of Coronavac, and 3 of Astrazeneca.

The median age of the students in the study was 16 years old, while the median age of employees was 47 years old. Most of the participants were female (55.5%). Thirty participants reported comorbidities (40% students and 60% employees) (table 1).

A total of 969 samples were tested, and the mean of testing was 108 tests per week (covering approximately 54% of individuals who agreed to participate in the study). During the study period, 322 symptomatic patients were tested by SCS public care municipality, and 40% of them were positive for COVID-19. Despite the presence of the virus in the municipality of SCS, none of the asymptomatic participants involved in the study tested positive for COVID-19 (Fig. 2).

At the 4th week of the study, one teacher had symptoms (October 20th). She was referred to a primary care clinic, and the RT-PCR test was positive for COVID-19. Simultaneously, 18 employees (9 collaborators and 9 professors) who had contact with the teacher during the previous days were tested for COVID-19, and all presented negative results.

Discussion

Our study pointed out that the respiratory symptoms surveillance system is a useful tool that can prevent the transmission of COVID-19 in a public school in a middle-income country such as Brazil. However, during the study period, the Delta variant, which is highly infective, was predominant in the state of São Paulo[9]; no cases of COVID-19 transmission occurred at school. All employees had been fully vaccinated with two doses of the COVID-19 vaccine, and students were vaccinated with at least one dose of the vaccine, which may have had an impact on the low positivity of the participants.

In Brazil, there was no clear policy of testing schools or the implementation of a national system of screening transmission of COVID-19 in schools. According to the 2020 census, Brazil has approximately 28,933 schools offering high school, representing 16.12% of total schools in the country(179,533)[10], and no free tests have been offered for public schools.

The saliva test has advantages such as self-collection, does not infer risk to participants and decreases the risk of exposure during sample collection. Studies have observed that the sensitivity of RT-LAMP in saliva varies from 85 to 95% in symptomatic patients[11,12 and that saliva has higher sensitivity than nasal/nasopharyngeal swab samples in asymptomatic patients[7].

The study has several limitations, was conducted in only one public school, and did not evaluate primary education children who are the population most at risk today for COVID-19 because they have not yet been vaccinated.

Conclusions

Implementation of an online system of respiratory symptom screening and testing of COVID-19 through saliva in asymptomatic individuals is a feasible, low-cost and practical option for monitoring and containing COVID-19, especially in low-income countries.

Declarations

Authors' contributions:

Oliveira, BA; Manuli, ER: Investigation, conceptualization, methodology, formal analysis and writing – original draft. Both authors contributed equally to the development of the study.

Leal, FE; Souza, ECB; Illi, AP; Passos-Bueno, MRS: Resources.

Paiva, APB; Barros, PL; Fachini, CS; Cano, BAM: Investigation.

Capuani, L; Domingues, HH: Analysis.

Sabino, EC; Costa, SF: Conceptualization, supervision and writing – review & editing. Both authors contributed equally to the development of the study.

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Competing interests:

The authors declare no competing interests.

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Table

Table 1. Participants characteristics of the respiratory symptoms screening system and testing of asymptomatic at a public school in Brazil

	Students	%	Employees	%	Total
Registered	431	85,2	75	14,8	506
Assent	141	70,5	59	29,5	200
Age years old (range)	15-21		21-73		
<i>Average</i>	16,6	-	48,3	-	26
<i>Median</i>	16	-	47	-	17
Sex					
<i>Male</i>	70	78,7	19	21,3	89
<i>Female</i>	71	64,0	40	36,0	111
Comorbidities					
<i>No information</i>	44	100,0	0	0,0	44
<i>No comorbities.</i>	85	74,6	29	25,4	114
<i>Comorbities</i>	12	40,0	18	60,0	30
<i>Cardiopatya</i>	2	15,4	11	84,6	13
<i>Cardiopatya & COPD*</i>	0	0,0	1	100,0	1
<i>Cardiopatya & Renal</i>	0	0,0	1	100,0	1
<i>Cardiopatya & Diabetes</i>	0	0,0	1	100,0	1
<i>Cardiopatya & Renal & Diabetes</i>	0	0,0	1	100,0	1

<i>COPD</i>	8	88,9	1	11,1	9
<i>COPD & Transplant</i>	0	0,0	1	100,0	1
<i>Renal Disease</i>	1	100,0	0	0,0	1
<i>Diabetes</i>	1	100,0	0	0,0	1
<i>Diabetes & Renal Disease</i>	0	0,0	1	100,0	1

*COPD: Chronic obstructive pulmonary disease

Figures

Figure 1. Diagram representation of participants included in the study.

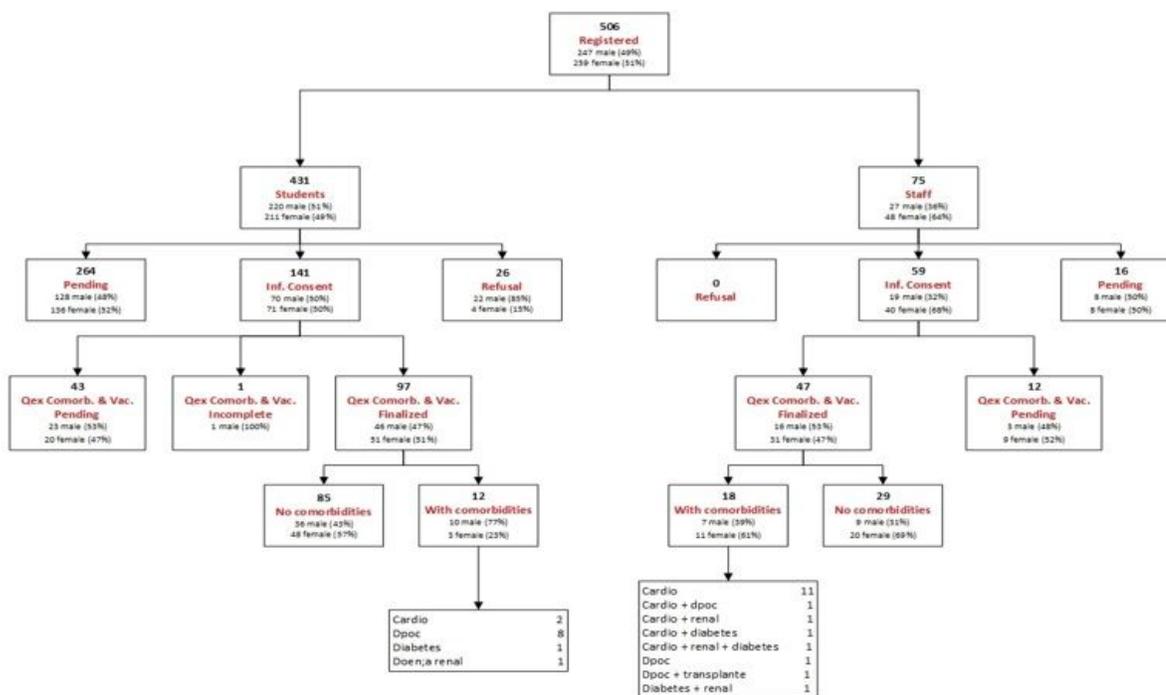


Figure 1

Diagram representation of participants included in the study.

Figure 2-Number of individuals tested at the São Caetano do Sul Municipality during the study period and asymptomatic participants tested for SARS-CoV-2 at the ONM public school.

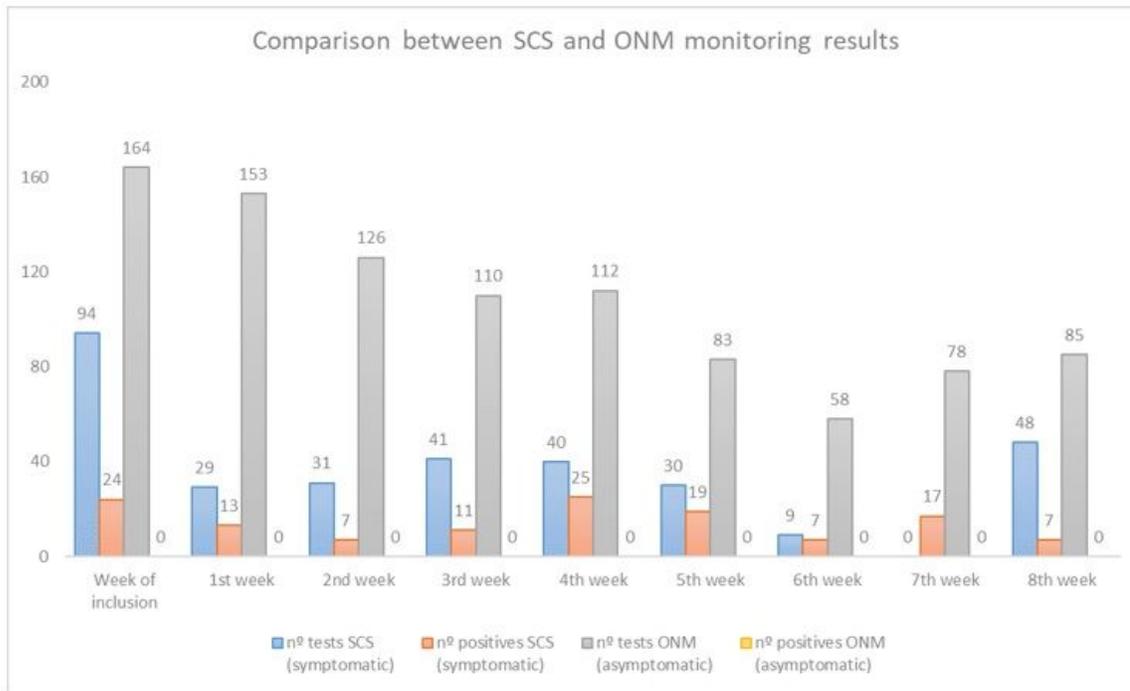


Figure 2

Number of individuals tested at the São Caetano do Sul Municipality during the study period and asymptomatic participants tested for SARS-CoV-2 at the ONM public school.