Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Population-based virucidal phthalocyanine gargle/rinse protocol to reduce the risk of coronavirus disease-2019: a community trial

Verônica Caroline Brito Reia (veronicabr@usp.br)
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0003-1352-5474
Roosevelt da Silva Bastos
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0001-5051-1210
Fabiano Vieira Vilhena
TRIALS – Oral Health & Technologies https://orcid.org/0000-0003-3840-3633
Heitor Marques Honório
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0003-0231-3409
Lucas Marques da Costa Alves
Hospital Estadual de Bauru https://orcid.org/0000-0001-9018-6395
Paulo Frazão
University of São Paulo https://orcid.org/0000-0002-3224-0020
Paulo Sérgio da Silva Santos
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0002-0674-3759

Research Article

Keywords: Coronavirus disease-2019, Severe acute respiratory syndrome coronavirus 2, Antiviral mouthwash, Epidemiology, Phthalocyanine

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1065834/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Purpose: In this community trial, the objective was to evaluate the incidence of coronavirus disease-2019 (COVID-19) cases in two similar communities in three distinct phases: phase 1 (before the intervention), 2 (during the intervention), and 3 (after the intervention).

Methods: The test community received the oral antiseptic intervention (experimental), while the control community did not. The official information agency provided the number of confirmed COVID-19 cases. Data were analyzed according to the three phases per epidemiological week (epi) using the R Core Team (2021) program. The relative risk and 95% confidence intervals between the cumulative incidence values of the test and control communities were calculated for each period. In the test community, a total of 995 residents, over 10 years of age, received two bottles containing 600 ml of mouthwash with antiviral phthalocyanine derivative (APD). The participants were asked to gargle/rinse with of 5 mL of the mouthwash with ADP 3 to 5 times a day, for 1 min, until the end of the bottles.

Results: In phases 1 and 3, disease risk between the two communities did not differ significantly ($p> 0.05$), while in phase 2, disease risk was 54% lower in the test community than in the control community.

Conclusion: The use of the APD mouthwash protocol seems to reduce the COVID-19 incidence at the population level, and further studies are needed to confirm its protective effect in different contexts.

Introduction

At the end of 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, and rapidly spread to other countries, initiating a pandemic period with high hospitalization and lethality caused by coronavirus disease – 2019 (COVID-19).[1–4] Faced with this situation, world leaders were expected to take primary prevention measures (health promotion and specific protection) to prevent the collapse of health systems. For this reason, the World Health Organization developed a strategic plan to block COVID-19 as follows: 1) mobilize all sectors and communities, 2) control sporadic cases and clusters and prevent community transmission, 3) suppress community transmission, 4) reduce mortality by providing appropriate clinical care for those affected by COVID 19 and 5) develop safe and effective vaccines and therapeutics.[4]

The protective effect of vaccines against SARS-CoV-2 has been assessed on the world population in order to prevent infections and symptoms, despite variants of the coronavirus.[4] However, wearing masks, washing hands, and social distance in conjunction with the use of mouthwashes has been recommended as an important preventive measure against the contamination and dissemination of SARS-CoV-2.[5–9] The recommendation of antiviral oral and nasal care products is associated with the knowledge that respiratory infections, such as COVID-19, favor the adhesion and replication of pathogens to the nasal and oral mucosa and, consequently, to the respiratory tract.[5] In this sense, the use of gargle/rinse protocols with oral antiseptic solutions has been indicated as an adjuvant therapy in the treatment of viral infections, as well as a prophylactic strategy to minimize the spread of the disease.[2, 5, 8, 10–12]

Phthalocyanine is an antimicrobial compound that acts by causing the selective destruction of viruses, bacteria, and other pathogens, with oxidizing potential and direct binding to the SARS-CoV-2 ribonucleic acid (RNA).[13] An anti-SARS-CoV-2 phthalocyanine derivative-containing mouthwash has been indicated during the pandemic, since COVID-19 positive patients showed clinical improvement and low severity after using a gargle/rinse protocol.[6–9]

Although the literature has reported the action of some antiviral mouthwashes to help against SARS-CoV-2,[12] no study has explored its effect on the incidence of COVID-19 in the populations. Here, we evaluated the action of an antiviral phthalocyanine derivative (APD) mouthwash for the risk reduction of COVID-19 in a Brazilian community.

Material And Methods

This controlled before-and-after community trial evaluated the cumulative incidence of COVID-19 cases over a period from 04/11/2020 to 05/15/2021 in two similar municipalities, Uru and Itaju, located in the Brazilian state with the highest number of accumulated cases. Both municipalities belong to the same mesoregion within São Paulo state, Brazil, and have a similar socio-demographic profile according to official data from the Brazilian Institute of Geography and Statistics.[14] The state of São Paulo is divided into 17 mesoregions, and COVID-19 was introduced at different times and behaved in different ways in each of them. The authors followed the epidemic curse in the mesoregion of Bauru, and the urban population living in Uru was invited to participate in the study in accordance with the principles of the Declaration of Helsinki, ethical standards of human experimentation, and biosafety protocols with the approval of the Human Research Ethics Committee (CAAE 39327120.3.0000.5417). It was also registered at the Brazilian Clinical Trial Register (RBR- 6c9xnw3). Uru was selected to receive the APD mouthwash protocol since this community had a major incidence of COVID-19 (> 50 cases/1,000 inhabitants) at the beginning of the study and because the health authority offered support promptly. To compare the estimates, we selected the population of Itaju as control that had a similar epidemic situation.

Setting and Municipalities epidemiological characteristics

The test and control communities included less than 5000 inhabitants, between 70 and 85% living in urban areas, with a demographic density of 7–15 inhab./km^2, municipal human development index = 0,7 and proportional labor force population within 15–30%. In addition, these two municipalities have only one access road and one public local health center (Figure 1).

With the spread of the SARS-CoV-2, some protective measures were adopted in the State of São Paulo government, followed strictly by Bauru Health Region authorities, as well as by both communities. Thus, quarantine measures were subdivided into four steps: Step 1 (red), considered a contamination period, allowed only essential services, in which commerce and schools were closed, and established mobility restrictions and mandatory social distance; step 2 (orange), considered a attention period with the possibility of allowing some services, such as commerce with occupation limitations of 20% and restriction of opening days adopting standard protocols, such as use of masks, alcohol gels, temperature measurement, and social distance; step 3 (yellow), considered a controlled period with some flexibility; and step 4 (green), considered a partial opening period, in which all services were allowed, respecting the 60% limit of occupancy, maintaining all specific protocols.[15–16]

Population Mouthwash Intervention Protocol

The test community included all the residents in the urban households over 10 years old, who after reading, understood the risks and objectives of the study, signed the informed consent form, and received two bottles containing 600mL of the APD mouthwash for use 2 months. They were instructed to use 5 mL of the mouthwash and to switch between gargling/rinsing 3 to 5 times, for 1 min during the day. The orientation was provided in writing and explained by the researcher with her research assistant for a period of 2 months, containing adequate information for the age groups of adults and children over 10 years old. During the period, the researcher was available to the population, who sought her out a few times to clarify doubts about the use of the product. Inhabitants who declared contraindications to using mouthwash for medical reasons or the inability to gargle and spit were excluded.

Data Sources and Outcome Measures

The study used official data from the Statewise System for Data Analysis maintained by the government of Sao Paulo state.[17] Information on cases and deaths referred to the place of residence of the patient. Consistency tests in the databases were performed daily, and their variations, characterized as outliers, were reported to the data collectors and reviewed in the calculations. Cases are considered confirmed for COVID-19 when a positive reverse-transcription polymerase chain reaction test result was obtained. The number of COVID-19 cases was divided into three phases according to the epidemiological week: 1-before the intervention (15th to 47th week); 2-during the intervention (48th week of 2020 to 4th week of 2021) and 3-after intervention (5th to 19th week).

Statistical Analysis

Data were organized in a Microsoft Office Excel 2016 spreadsheet (Microsoft Corporation, Redmond, WA, USA), and analyses were conducted using the R Core Team program (2021). Incidences and risks of the disease in the three phases described for the control and test communities were calculated. Considering the size of populations under comparison, the relative risk (RR), standard error, and 95% confidence interval were estimated by subtracting the confirmed cases of the earlier phase in the denominator of the reference population. Differences at the 10% level were assumed to be significant because of the innovative features of the study. The null hypothesis (H0) proposed that there was no difference between the two municipalities regarding the RR.

Results

Figure 2 illustrates the total number of confirmed cases of COVID-19 for the test and control communities before, during, and after the intervention. Figure 3 shows the curves of cumulative cases in each community for every 3 epidemiological weeks. The gray area is related to the intervention period (phase 2). In phase 1, the increase in new cases began earlier in the control community (26th week of 2020) than in the test community (32nd week of 2020); however, since the 35th week of 2020, cumulative cases in the test community overcame those in the control community. In phase 2, the number of new cases increased more in the control community than in the test community. In phase 3, after the intervention period, the increase in new cumulative cases was similar between the 4th and 16th week of 2021. The curves of cumulative incidence of each community patterned every 3 weeks showed small differences in deflection related to the dynamics of epidemics in each community. It is noteworthy that the test community showed a different pattern in the intervention period (gray area of Figure 3) and that both communities' curves met each other at the end of the entire observation period.

In the period prior to the intervention (phase 1), the RR between the two municipalities was 1.15 (0.87–1.51) and did not significantly differ. The cumulative incidence of the test community was 54.6 per 1,000 residents, while it was 47.6 in the control community ($p=0.331$). In the intervention period (phase 2), the RR between the municipalities was 0.46 (0.20–1.08) with a statistically significant reduction in risk (-54.0%). The cumulative incidence in the test community was 5.2% per 1,000 residents, while in the control community it was 11.3% ($p=0.076$). In the period after the intervention (phase 3), the RR was 0.92 (0.69–1.23) and the cumulative incidences did not differ between the test and control communities ($p=0.591$) (Table 1).

Table 1
– Cumulative incidence values and relative risk of coronavirus disease-2019 cases between the test and control communities in each evaluated period

	Period	Number of weeks in the period	Confirmed cases	Cumulative incidence*	Relative risk (95%CI)	Variation in risk	p-value
Control	1 (before)	33	185	47.6	Reference		
Test	1 (before)	33	63	54.6	1.15 (0.87–1.51)	+15.0%	0.331
Control	2 (during)	9	44	11.3	Reference		
Test	2 (during)	9	6	5.2	0.46 (0.20–1.08)	-54.0%	**0.076**
Control	3 (after)	15	208	53.5	Reference		
Test	3 (after)	15	57	49.4	0.92 (0.69–1.23)	-8.0%	0.591
Control	Total	57	437	112.4	Reference		
Test	Total	57	126	109.3	0.97 (0.81–1.17)	-3.0%	0.766

*Cases per 1,000 population. CI, confidence interval

Discussion

The main finding of this study was that the cumulative incidence of COVID-19 in the test community was lower than that in the control community during the use of a population-based virucidal phthalocyanine gargle/rinse protocol. This is a promising result, as COVID-19 is very recent, and there is still no "gold standard" treatment; thus, actions based on primary prevention measures to slow down and interrupt transmission are essential. Populations pre-experienced in viral epidemics adopted preventive measures with good risks and benefits, which is defended, such as social distancing, washing hands, wearing masks, and gargling/rinsing with antiseptic solutions.[18–21] Since saliva was an important source of virus transmission during the pandemic, protective measures, such as the use of antiseptic gargles were adopted and recommended by several countries and organizations against SARS-CoV-2.[5, 10, 21–23]

Some studies have shown that the use of a mouthwash containing APD can reduce the viral load of SARS-CoV-2, acting as a therapeutic aid in reducing the severity and risk of COVID-19 transmission. [8–9,] [22–24] These data corroborate the findings of the present study. The municipalities divided as test and control did not differ in terms of disease risk before the period of distribution of mouthwashes (phase 1) ($p>0.05$); however, disease risk was lower in the test than in the control community after the widespread use of the mouthwash/gargling protocol with APD.

COVID-19 is a disease with pandemic characteristics that spreads according to population mobility.[16] The municipalities selected in this community trial had similar population sizes and social indicators. They belonged to the same geographic mesioregion, had a similar structure to primary healthcare, and were connected by a single route with neighboring municipalities.[25–26] In both communities, the measures to control the COVID-19 pandemic in the whole study period were similar regarding the recommendations for social distance and self-administered hygiene measures inducing people to wear face masks, frequently wash their hands with soaps, and, if necessary, use alcohol gel on the hands. Therefore, there were no reasons for any incidence difference in COVID-19 cases from both communities, except for the virucidal phthalocyanine gargle/rinse protocol used in the test community. Owing to these points, the comparison presented herein is assumed to be licit.

The cumulative incidence difference of COVID-19 at the population level in the test community was nearly significant, showing promising findings. Prior to the beginning of the use of the APD mouthwash the cumulative COVID-19 cases were visually similar; however, at the 38th week, the test community's curve seemed to deflect differently with a small number of new cases, while still showing a slight increase. However, despite the control community continuing to increase the COVID-19 cases, in the test community, new cumulative cases per 1,000 inhabitants were lower than those in the control community. In phase 2, the moment the population was exposure to the APD mouthwash, the relative risk decreased to 0.46, suggesting a preventive effect in the test community. The p-value was borderline ($p=0.076$) to significant, sufficient to be considerable in an epidemiologic study in a very difficult history moment. After phase 2, both communities increased the number of new COVID-19 cases. After the intervention period, the test community data returned to the new cumulative case curve similar to the control community, reinforcing the potential effect of the APD mouthwash at the population level.

Saliva is a source of biological fluid in the spread of the disease COVID-19. The presence of SARS-CoV-2 in saliva causes viral proliferation and consequent RNA secretion in any cells involved in the production of salivary components, such as salivary glands, respiratory tract cells, and the periodontal tissue.[27–28] Although our research did not analyze the salivary components and substantivity, we observed a difference in COVID-19 risk in the test community compared to the control community. This was supported by the finding that the virus has been consistently detected in saliva; thus, the oral cavity was a source of SARS-CoV-2.[29] Thus, rinsing/gargling with an APD solution could reduce the chemical/mechanical action of the virus from the oral cavity and throat. In addition, a chemical antiviral aerosol can be generated and inhaled to protect the upper airways when a gargle is done.

Strengths and limitations of this study must be considered in relation to the methodology used. The study was undertaken in an adverse historic moment, and the field team did not have the conditions to register the use of the APD mouthwash. Thus, important variables, such as the personal/family's behavior related to COVID-19 could not be obtained to control for possible confounding factors. On the opposite, all the assessments were conducted at the population level, without any interference from the research group regarding each case and were publicly registered in both communities. It is noteworthy that the results were observed in the Brazilian context in which the testing strategy was restricted. The widespread use of masks, which could have also contributed to reducing the transmissibility of COVID-19, was not assessed in this study. A difference in COVID-19 cumulative cases was observed in the current study, suggesting an effect of the intervention and possible contribution to prevent the health system from collapsing while preserving lives.

Conclusion

The use of the APD mouthwash protocol seems to reduce the COVID-19 incidence at the population level, and further studies are needed to confirm its protective effect in different contexts.

Declarations

Funding source: Funding This research was funded primarily by TRIALS – Oral Health & Technologies. Funders contributed to the scope and design of this study; however, they did not influence the collation, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (Finance Code 001). Dr. Da Silva Santos reports from CNPq process nº. 309525/2018-7. Dr. Frazão received scholarship from CNPq process nº. 305132/2019-9.

Conflict of Interest: The authors declare no conflicts of interest.

Acknowledgments: To TRIALS – Oral Health & Technologies and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. To Magali de Brito, research assistant the study.

References

1. - Gao Z, Xu Y, Sun C, et al. A Systematic Review of Asymptomatic Infections with COVID- 19. *J Microbiol Immunol Infect*, 54(1):12–16 (2021). https://doi.org/10.1016/j.jmii.2020.05.001.
2. - Peng X, Xu X, Li Y, et al. Transmission routes of 2019-nCoV and controls in dental practice. *Int J Oral Sci.*, 2020;12,9. https://doi.org/10.1038/s41368-020-0075-9
3. - Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*, 2020;579, 265–269. https://doi.org/10.1038/s41586-020-2008-3.
4. - World Health Organization et al. Coronavirus disease (COVID-19): situation report, 2020; p.137. https://www.who.int/docs/default-source/coronaviruse/covid-strategy-update-14april2020.pdf.

5. - Kramer A, Eggers M, Hübner NO, et al. Virucidal gargling and virucidal nasal spray. *GMS Hyg Infect Control.* 2021;16:Doc02. Published 2021 Jan 18. https://doi.org/10.3205/dgkh000373.
6. - Orcina B. F & Santos, P.S S S. Oral Manifestation COVID-19 and the Rapid Resolution of Symptoms Post-Phtalox Treatment: a Case Series. *Int. J. Odontostomat.* 2021;15(1):67–70.
7. - da Fonseca Orcina B, Vilhena FV, Cardoso de Oliveira R, et al. A Phthalocyanine Derivate Mouthwash to Gargling/Rinsing as an Option to Reduce Clinical Symptoms of COVID-19: Case Series. *Clin Cosmet Investig Dent.*, 2021;13:47–50. Published 2021 Feb 18. https://doi.org/10.2147/CCIDE.S295423.
8. - Santos C, da Fonseca Orcina B, Brito Reia VC, et al. Virucidal Activity of the Antiseptic Mouthwash and Dental Gel Containing Anionic Phthalocyanine Derivative: In vitro Study. *Clin Cosmet Investig Dent.*, 2021;13:269–74. https://doi.org/10.2147/CCIDE.S315419.
9. - Vilhena FV, Brito Reia VC, da Fonseca Orcina B, et al. The use of antiviral Phthalocyanine mouthwash as a preventive measure against COVID-19. GMS *Hyg Infect Control.*, 2021 Jul 9;16:Doc24. https://doi.org/10.3205/dgkh000395.
10. - Carrouel F, Conte M. P, Fisher J, et al. COVID-19: A Recommendation to Examine the Effect of Mouthrinses with β-Cyclodextrin Combined with Citrox in Preventing Infection and Progression. *J Clin Med.*, 2020 Apr 15;9(4):1126. https://doi.org/10.3390/jcm9041126.
11. - Nagatake T, Ahmed K, Oishi K. Prevention of Respiratory Infections by Povidone-Iodine Gargle. *Dermatology, Basel.* 2002;204 Suppl 1:32–6. https://doi.org/10.1159/000057722.
12. - Mateos-Moreno MV, Mira A, Ausina-Márquez V, Ferrer MD. Oral antiseptics against coronavirus: in-vitro and clinical evidence. *J Hosp Infect.*, 2021 Jul;113:30–43. https://doi.org/10.1016/j.jhin.2021.04.004.
13. - Encinar JA, Menendez JA. Potential Drugs Targeting Early Innate Immune Evasion of SARS-Coronavirus 2 via 2'-O-Methylation of Viral RNA. *Viruses*, 2020 May 10;12(5):525. https://doi.org/10.3390/v12050525.
14. - Instituto Brasileiro de Geografia e Estatística (IBGE), 2020. https://cidades.ibge.gov.br/brasil/sp/uru/panorama.
15. - De Almeida GB, Pronunciate M, Grotto R, et al. Two hundred days of COVID-19 in São Paulo State, Brazil. *Epidemiol and Infect.*, 2020 Dec 2;148:e295.. https://doi.org/10.1017/S0950268820002927.
16. - Fortaleza CMCB, Guimarães RB, Catão RC, et al. The use of health geography modeling to understand early dispersion of COVID-19 in São Paulo, Brazil. *PLoS ONE*, 2021;16(1):e0245051. https://doi.org/10.1371/journal.pone.0245051.
17. - Fundação Sistema Estadual de Análise de Dados (SEADE), 2021. https://www.seade.gov.br/coronavirus/#.
18. - Tashiro A and Shaw R. COVID-19 Pandemic Response in Japan: What Is behind the Initial Flattening of the Curve? *Sustainability*, 2020;12(13):5250. https://doi.org/10.3390/su12135250.
19. - Vietnam Health Environment Management Agency (VIHEMA). COMMUNICATION ON DISEASE PREVENTION AND CONTROL - COVID-19 [Internet]. [cited 2021 feb 12]. Available at

https://vihema.gov.vn.

20. - Konishi Y, Saito T, Ishikawa T, et al. How Did Japan Cope with COVID-19? Big Data and Purchasing Behavior. *Asian Economic Papers*, 2021;20(1):146–167. https://doi.org/10.1162/asep_a_00797.
21. - Eduardo FP, Corrêa L, Heller D, et al. Salivary SARS-CoV-2 load reduction with mouthwash use: A randomized pilot clinical trial. *Heliyon*, 2021;7(6):e07346. https://doi.org/10.1016/j.heliyon.2021.e07346.
22. - da Silva Santos PS, da Fonseca Orcina B, da Costa Alves LM, et al. A Recommendation of PHTALOX® Mouthwash for Preventing Infection and Progression of COVID-19. *Acta Scient Dent Sci*, 2020;4(12):111–2. https://doi.org/10.31080/ASDS.2020.04.0991.
23. - da Silva Santos PS, da Fonseca Orcina B, Machado RRG. et al. Beneficial effects of a mouthwash containing an antiviral phthalocyanine derivative on the length of hospital stay for COVID-19: randomised trial. *Sci Rep.*, 2021;11,19937. https://doi.org/10.1038/s41598-021-99013-5.
24. - Carrouel F, Gonçalves LS, Conte MP, et al. Antiviral Activity of Reagents in Mouth Rinses against SARS-CoV-2. *J Dent Res.*, 2021 Feb;100(2):124–32. https://doi.org/10.1177/0022034520967933.
25. - Casale M, Rinaldi V, Sabatino L, et al. Could nasal irrigation and oral rinse reduce the risk for COVID-19 infection?. *Int J Immunopathol Pharmacol,.* 2020;34:2058738420941757. https://doi.org/10.1177/2058738420941757.
26. - Burton MJ, Clarkson JE, Goulao B, et al. Antimicrobial mouthwashes (gargling) and nasal sprays administered to patients with suspected or confirmed COVID-19 infection to improve patient outcomes and to protect healthcare workers treating them. *Cochrane Database Syst Rev.*, 2020 Sep 16;9(9):CD013627. https://doi.org/10.1002/14651858.CD013627.pub2.
27. - Matuck, BF, Dolhnikoff M, Duarte-Neto AN, et al. Salivary glands are a target for SARS-CoV-2: a source for saliva contamination. *J. Pathol.* 2021;254: 239–243. https://doi.org/10.1002/path.5679.
28. - Fernandes Matuck B, Dolhnikoff M, Maia GVA, et al. Periodontal tissues are targets for Sars-Cov-2: a post-mortem study. *J Oral Microbiol.*, 2020 Nov 26;13(1):1848135. https://doi.org/10.1080/20002297.2020.1848135.
29. - To KK, Tsang OT, Yip CC, et al. Consistent Detection of 2019 Novel Coronavirus in Saliva. *Clin Infect Dis.*, 2020;71(15):841–843. https://doi.org/10.1093/cid/ciaa149.

Figures

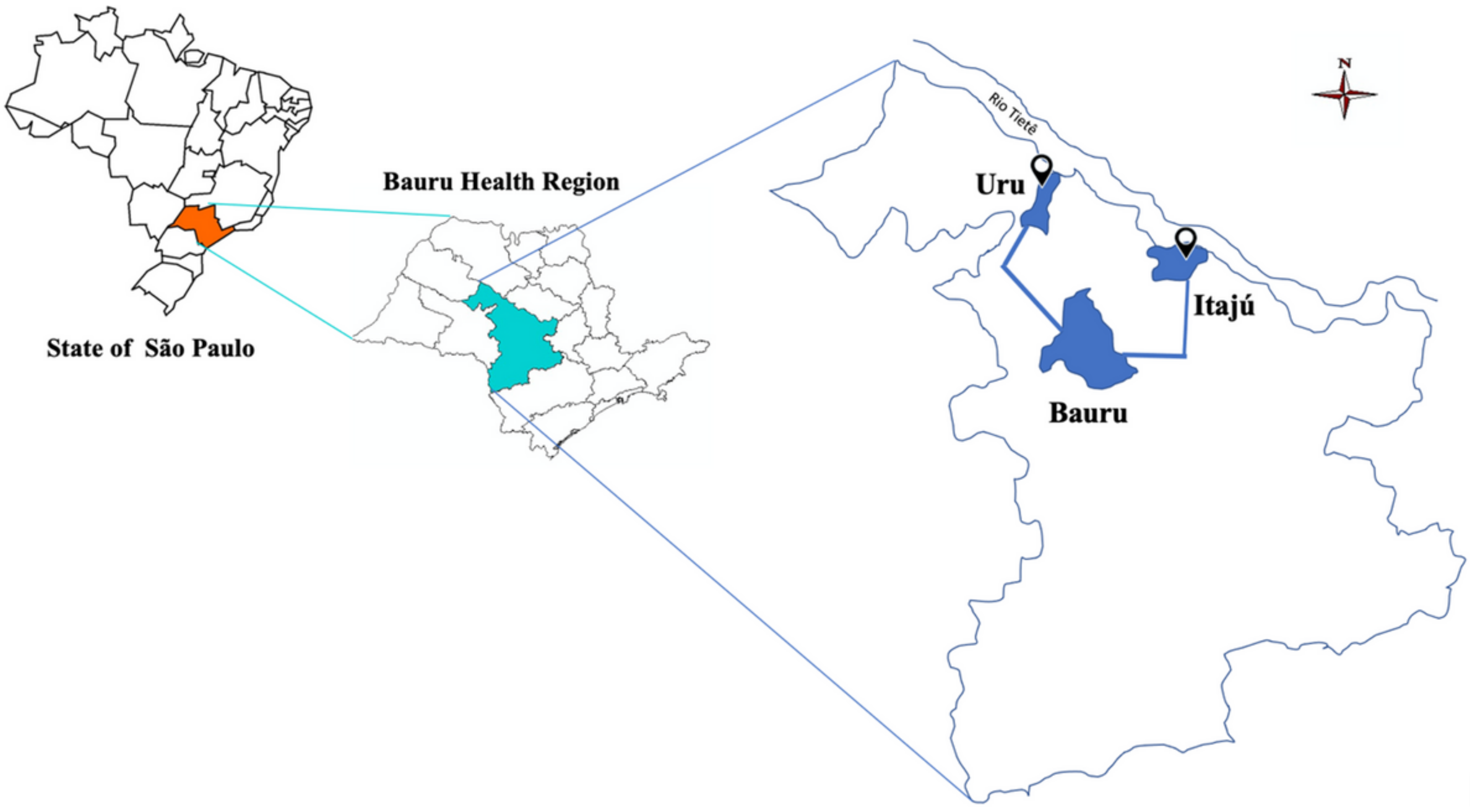

Figure 1

Map of the Bauru regional health, test and control community. Adapted from Seade foundation (SEADE).

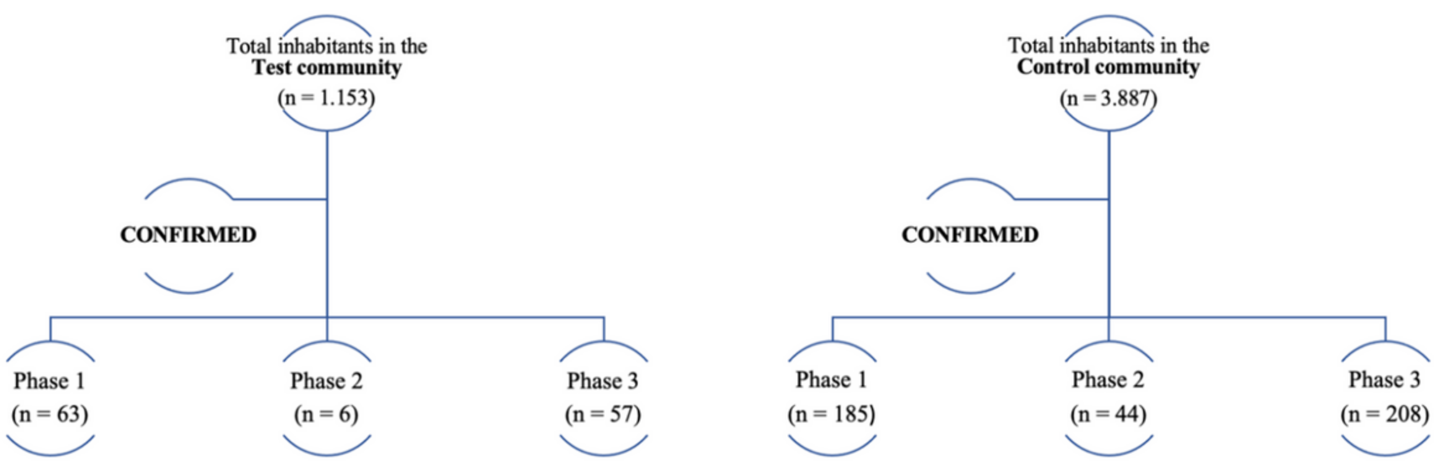

Figure 2

Flowchart of the reference population.

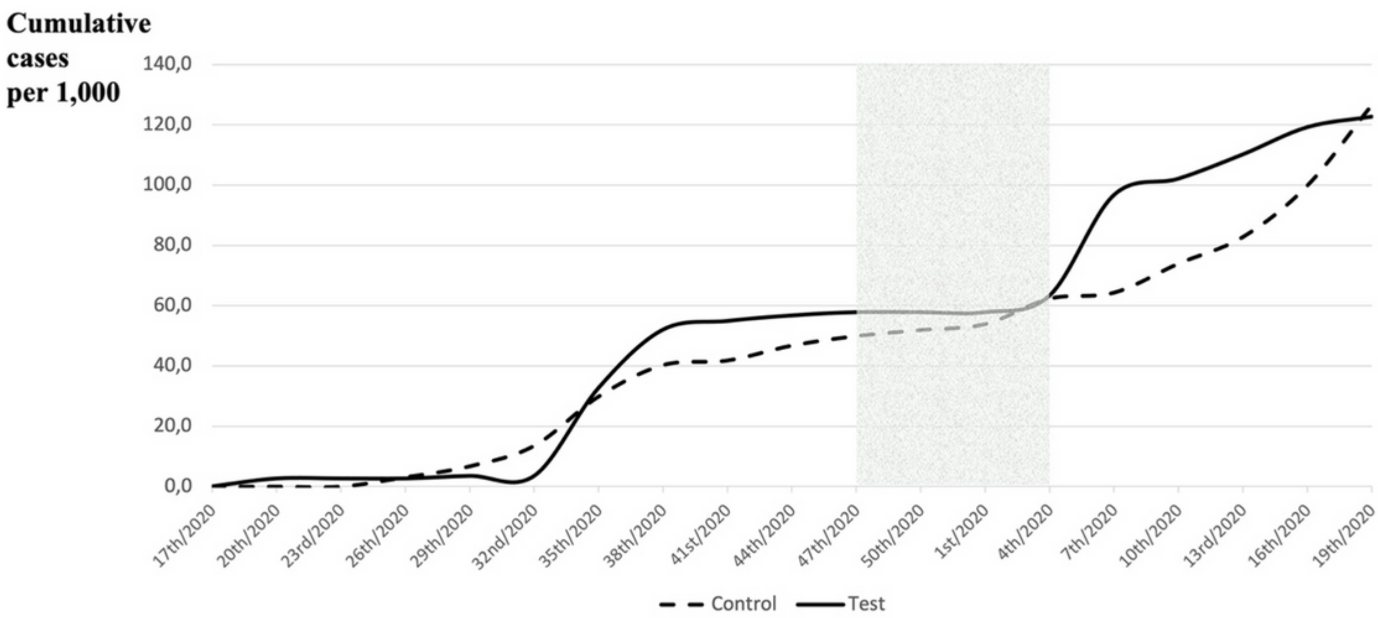

Figure 3

Cumulative incidence per 1,000 inhabitants in the test and control communities every 3 epidemiological weeks.