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## Analysis of testing for COVID-19 in Parnaíba city, state of Piauí, from march to December 2020

*Análise da testagem para COVID-19 na cidade de Parnaíba, estado do Piauí, de março a dezembro de 2020*

*Análisis de pruebas de COVID-19 en la ciudad de Parnaíba, estado de Piauí, de marzo a diciembre de 2020*

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### ABSTRACT

**Background and Objectives:** it is extremely important and necessary to assess epidemiological events through the analysis of measures adopted at the time of crises, especially those with a health impact as a way of improving the system for future events, with testing being a gold standard to be assessed during an epidemic. This study aimed to analyze tests for COVID-19 diagnosis, with a view to detecting possible false negative results, in Parnaíba, Piauí, from March to December 2020. **Methods:** a statistical analysis of the data reported and made available by the Municipal Health Department using the IBM SPSS Statistics<sup>®</sup> 21.0 software, in which the variables type of test, date of symptom onset and date of material collection were crossed to obtain the results. **Results:** a total of 9,473 tests were negative, of which 11.1% were carried out using the RT-PCR methodology, 6.5% using rapid antigen tests, and 82.3% using a rapid antibody test. The analysis revealed that only 0.47% RT-PCR tests and 1.7% rapid antigen tests had been carried out within the ideal testing interval. On the other hand, the rapid antibody test had 0.14% performed outside the range. **Conclusion:** the most successful diagnostic test was the rapid antibody test, but it is the least specific and not suitable for determining health crisis management policies, especially for isolation measures for infected people, which suggests improvements in testing systems and development of tests with longer and more accurate testing intervals.

**Keywords:** SARS-CoV-2. Epidemiology. Clinical Laboratorial Techniques. Public Health.

### RESUMO

**Justificativa e Objetivos:** é de suma importância e necessidade a avaliação dos eventos epidemiológicos por meio da análise das medidas adotadas no momento das crises, em especial aqueles de impacto sanitário como forma

de melhor o sistema para eventos futuros, sendo a testagem um padrão-ouro a ser avaliado durante uma epidemia. O objetivo deste estudo foi analisar os testes para o diagnóstico de COVID-19, na perspectiva de detectar possíveis resultados falsos negativos, em Parnaíba, Piauí, de março a dezembro de 2020. **Métodos:** análise estatística dos dados notificados e disponibilizados pela Secretaria Municipal de Saúde a partir do *software* IBM SPSS® Statistics 21.0, em que as variáveis tipo de teste, data do início dos sintomas e data da coleta do material foram cruzadas para obtenção dos resultados. **Resultados:** 9.473 testes resultaram negativo, em que 11,1% foram realizados pela metodologia RT-PCR, 6,5%, pelos testes rápidos de antígeno, e 82,3%, por teste rápido de anticorpo. A análise revelou que apenas 0,47% testes por RT-PCR e 1,7% testes rápidos de antígeno haviam sido realizados dentro do intervalo ideal de testagem. Por outro lado, o teste rápido de anticorpo teve 0,14% realizados fora do intervalo. **Conclusão:** o teste com maior sucesso de diagnóstico foi o teste rápido de anticorpo, porém é o menos específico e não adequado para determinação de políticas de gerenciamento de crise sanitária, em especial para medidas de isolamento de infectados, o que sugere melhorias em sistemas de testagem e desenvolvimento de testes com intervalos de testagem maior e precisos.

**Descritores:** SARS-CoV-2. Epidemiologia. Diagnóstico Laboratorial. Saúde Pública.

## RESUMEN

**Justificación y Objetivo:** es sumamente importante y necesario evaluar los eventos epidemiológicos a través del análisis de las medidas adoptadas en el momento de las crisis, especialmente aquellas con impacto en la salud, como una forma de mejorar el sistema para eventos futuros, siendo las pruebas un estándar de oro a ser evaluado durante una epidemia. El objetivo de este estudio fue analizar pruebas para el diagnóstico de COVID-19, con miras a detectar posibles resultados falsos negativos, en Parnaíba, Piauí, de marzo a diciembre de 2020. **Métodos:** análisis estadístico de los datos reportados y puestos a disposición por la Secretaría de Salud Municipal mediante el *software* IBM SPSS® Statistics 21.0, en el cual se cruzaron las variables tipo de prueba, fecha de inicio de síntomas y fecha de recolección del material para obtener los resultados. **Resultados:** un total de 9.473 pruebas resultaron negativas, de las cuales el 11,1% se realizaron mediante la metodología RT-PCR, el 6,5% mediante pruebas rápidas de antígenos y el 82,3% mediante prueba rápida de anticuerpos. El análisis reveló que sólo el 0,47% de las pruebas RT-PCR y el 1,7% de las pruebas rápidas de antígenos se habían realizado dentro del intervalo de prueba ideal. Por otro lado, la prueba rápida de anticuerpos tuvo un 0,14% realizado fuera del rango. **Conclusión:** la prueba diagnóstica más exitosa fue la prueba rápida de anticuerpos, pero es la menos específica y no adecuada para determinar políticas de gestión de crisis sanitarias, especialmente para medidas de aislamiento de personas infectadas, lo que sugiere mejoras en los sistemas de pruebas y desarrollo de pruebas con tiempos más largos y precisos. intervalos de prueba.

**Palabras Clave:** SARS-CoV-2. Epidemiología. Técnicas de Laboratorio Clínico. Salud Pública.

## INTRODUCTION

COVID-19 was first reported in the Chinese province of Wuhan in 2019. From then on, the number of infected cases grew on a global scale, which is why it was classified by the World Health Organization (WHO) as a health emergency public interest of international concern.<sup>1</sup>

Caused by SARS-CoV-2, COVID-19 is a disease with a varied clinical appearance, and can present from asymptomatic to severe conditions leading to death. Depending on the emergence of the first and subsequent cases, approximately 80% of patients are asymptomatic and 20% of detected cases require hospital care.<sup>1</sup>

The clinical picture of the disease occurs after an incubation period of between two and 14 days, and the most common symptoms are dry cough, fever, dyspnea, headache, myalgia, fatigue and diarrhea.<sup>2</sup> Acute respiratory distress syndrome (ARDS) is one of the most serious complications, associated with prolonged hospitalization and high mortality.<sup>3</sup>

Moderate and severe cases require hospitalization with drug therapy with antipyretics, antivirals, antibiotics and steroids.<sup>23</sup> The advent of the vaccine and its complete progression in adults has meant that severe cases of

COVID-19 tend to concentrate in unvaccinated populations.<sup>4</sup>

The gold standard diagnosis for identifying the SARS-CoV-2 virus is made using reverse transcription polymerase chain reaction (RT-PCR) with real-time amplification, and, for molecular identification of the variant, partial or total sequencing of the viral genome is necessary. RT-PCR depends on the reverse transcriptase enzyme, which specifically amplifies the fragment of interest. When testing for the virus, the first complementary DNA (cDNA) is synthesized using reverse transcriptase followed by polymerase chain reaction (PCR). This offers greater sensitivity and specificity than nucleic acid tests.<sup>5</sup>

Ideally, collection should be carried out after the appearance of symptoms, between the third and fifth day and up to seven days after the event, since in samples collected early or late, false negative results can be obtained, and the same may occur with insufficient material collection methodology from the nasopharynx or contaminated samples.<sup>6</sup>

Like all other viral infections, the body reacts to the presence of the virus by producing antibodies, initially of the immunoglobulin A (IgA) class, followed by immunoglobulin M (IgM) and immunoglobulin G (IgG). Sero-

logical testing can be implemented using two different techniques: ELISA and immunochromatographic assays. Most COVID-19 patients begin producing antibodies between seven and 11 days after exposure to the virus, although some may develop antibodies sooner.<sup>7</sup> Rapid tests are the types of serological tests that detect antibodies produced upon exposure to the virus. Instead, they could also be based on the detection of antigenic viral proteins in patient samples. They are less sensitive than nucleic acid-based tests.<sup>8</sup>

The rapid antigen test, a test that emerged later, is an immunochromatographic assay that qualitatively detects SARS-CoV-2 antigens against the infection, and must be performed between the second and seventh day of the onset of symptoms. Its performance does not require complex structures or specialized devices, most of the time generating a rapid diagnostic response, which helps prevent vertical transmission of the disease.<sup>9</sup>

In addition to the availability and distribution of vaccines, local, social and demographic characteristics must be taken into account in response strategies to the epidemic, since the country has a large population, distributed unevenly across the territory, with cultural and cultural differences. geographical areas that can influence adherence to interventions, in addition to showing marked social inequalities and in access to health services.<sup>9</sup>

In cases of health crises, the priority is to establish the profile of the infection by recognizing the etiological agent and testing the population so that it is possible to outline combat and prevention measures. Analyzing testing is a way of assessing the combat measures adopted and generating knowledge on how to act in future situations. The study took place with the purpose of analyzing tests for COVID-19 diagnosis, with a view to detecting possible false negative results in the state of Piauí, with data reported to the Municipal Health Department through Epidemiological Surveillance, available for notification to the Ministry of Health (MoH).

## METHODS

This is a descriptive epidemiological study. The research was carried out with epidemiological data from the city of Parnaíba, state of Piauí, Northeast region of the country, collected at the Municipal Health Surveillance Department, covering the period from March to December 2020, tabulated in Microsoft® Office Excel 2010 software. for Microsoft® Windows 10. These were transferred to the researchers in their original format in Microsoft® Office Excel 2010 and then exported to IBM SPSS® Statistics software. Data were described with sociodemographic (gender, age, city of residence) and clinical-epidemiological variables (date of notification, type and result of the test, date of onset of symptoms, date of testing, symptoms, associated disease conditions pre-existing conditions and description of symptoms).

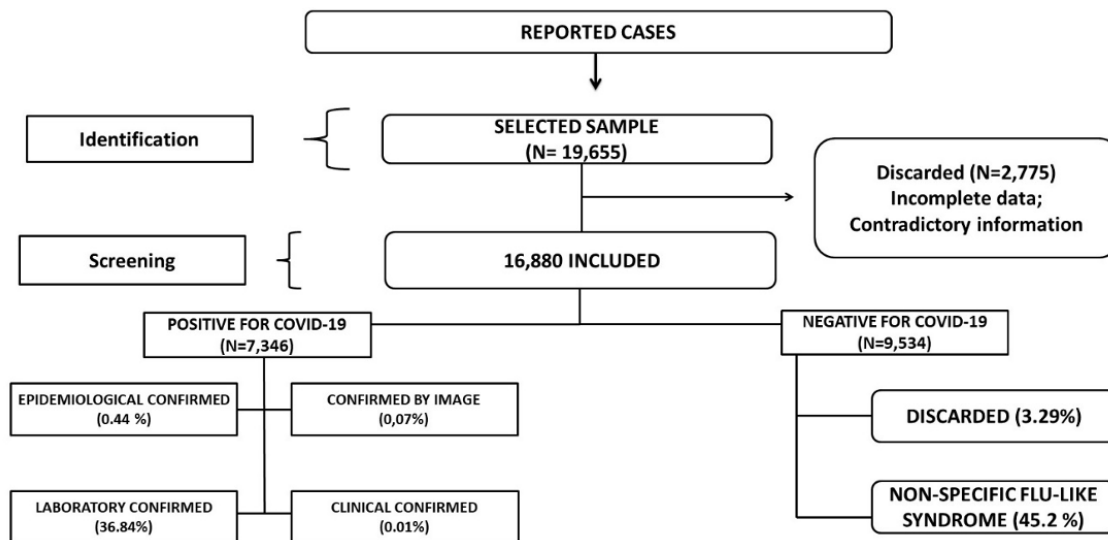
In Microsoft® Office Excel 2010, the data was sorted, and those that were incomplete or had typing errors that compromised understanding for data analysis were excluded from the study. To have an overview of diagnosis, data confirmed by clinical-epidemiological criteria and diagnostic imaging were initially considered only as complementary information, being excluded in statistical analyses, as they are non-specific for diagnosis. Negative data were classified as discarded and non-specific flu-like syndromes, with those tested using RT-PCR or serology methods being selected, followed by import into IBM SPSS® Statistics 21.0, for statistical analysis and obtaining results through graphs and tables.

For data analysis, the time intervals from the onset of symptoms to the test were classified using the terms "adequate", when they comply with the test usage interval, or "inadequate", when they do not comply with, following the MoH recommendations based on the Epidemiological Surveillance guide, which recommends the ideal intervals and tests for an accurate diagnosis, with parameters for appropriate intervals after the onset of symptoms: RT-PCR test, for an interval of three to seven days; rapid antibody test, for intervals  $\geq$  eight days; rapid antigen test, for an interval of two to seven days; ELISA IgM and IgG test, for intervals  $\geq$  eight days. Tests performed outside these standards will be classified as inadequate.

After data analysis, 2,775 cases were excluded, with the exclusion criterion being the unavailability of complete data, making them invalid for the study. The remaining quantity was 16,880 suitable tests, of which 7,346 totaled positive results with a higher percentage of 36.84%, confirmed by laboratory criteria. On the other hand, 9,534 was the total number of negative cases, of which 3.29% were ruled out for COVID-19 and 45.2% were classified as non-specific flu-like syndrome (Figure 1).

After classification, the mean and standard deviation of the time intervals between the onset of symptoms and the tests were calculated according to adequacy classification. Variables were summarized as medians and interquartile ranges, while categorical variables were expressed as counts and percentages. Odds Ratios (OR) with 95% Confidence Intervals were calculated to identify risk factors in univariate logistic regression models. After obtaining statistical results, graphs and tables were generated in the present study.

All ethical precepts established by Resolution 466/2012 of the Brazilian National Health Council (CNS – *Conselho Nacional de Saúde*) were respected with regard to ensuring information legitimacy, privacy and confidentiality, making the results of this research public, when necessary. The research presents the substantiated opinion of the Research Ethics Committee, under the Certificate of Presentation for Ethical Consideration (CAAE - *Certificado de Apresentação para Apreciação Ética*) 52834021.0.0000.0192, approved on December 6, 2021, under Opinion 5.147.515.



**Figure 1.** Representative scheme of reports for detecting positive cases for COVID-19 through clinical-laboratory confirmation during the second half of 2020 following the methodological sequence of the study.

## RESULTS

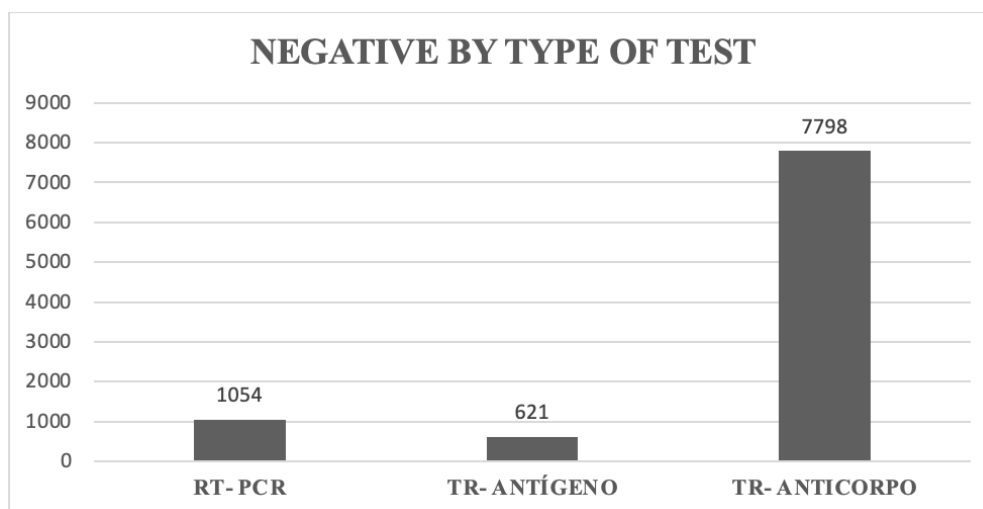
Due to the initial challenges of the pandemic, which particularly hampered the notification and testing of cases, samples from the first half of 2020 were excluded and samples from the second half of 2020 were selected, as it is the interval in which knowledge of disease patterns and the establishment of procedures that constitute greater availability of complete and more solid data were better developed, totaling 19,655 cases reported in the city of Parnaíba, state of Piauí, in this period of time.

The tests used in diagnoses with negative results were 1,054 using the RT-PCR methodology, 621 using the rapid antigen test, and 7,798 using the rapid antibody test (IgG and IgM) (Figure 2).

Among the three main tests used in the period, it was observed that, among those tested using the RT-PCR methodology, representing approximately 11.1% of those

tested with a negative result, 1,049 were carried out outside the recommended interval of three to seven days for testing, which represented 99.5% of tests using this methodology with possible false negatives (Table 1). The rapid antigen test presented a similar statistic, in which 610 of its 621 tests did not respect the ideal methodological intervals for testing, which characterizes a test with 98.2% of negative results with characteristics of possible false negatives, with these intervals being recommended as a basis for a more accurate diagnosis and with greater sensitivity and specificity of these methodologies.

The test performance and result vary depending on circumstances such as sample storage or transport, serum conversion and decline in antibody titer as well as testing interval. Therefore, obtaining diagnostic tests with high sensitivity and specificity, combined with appropriate analytical conditions for sample collection and treatment, avoids a high frequency of false negative



**Figure 2.** Results of negative cases tested by RT-PCR methodologies, and rapid antigen and antibody tests, referring to the second half of 2020.

results that can influence infection control strategies.

On the other hand, the rapid antibody test presented a result with interesting methodological errors, presenting only 11 of its 7,798 tested inappropriately, which elects it as one of the tests with the least false negatives, since the testing error was less than 1%, but its result is already expected when taking into account its applied methodology, discussed in this study.

An important point observed was that, according to the notification data, all tests carried out followed the same parameter. Most patients tested had more than ten days of symptoms.

**Table 1.** Quantity of tests during the study period and the list of total tests carried out outside the testing interval classified as inadequate following the Ministry of Health and test manufacturers recommendations.

TYPE OF TEST	FULLY TESTED N (%)	INADEQUATE TESTING N (%)
RT-PCR	1.054 (11.1)	1.049 (99.5)
RT-ANTIGEN	621 (6.5)	610 (98.2)
RT-ANTIBODY	7.798 (82.3)	11 (0.14)

## DISCUSSION

The pandemic has highlighted variation in access to healthcare, healthcare infrastructure and preparedness across regions, and these in turn have significantly affected outcomes. The accuracy of official data in the first months of the pandemic was quite challenging, a moment that, despite the efforts of health services, which were overloaded, to notify, operational difficulties, laboratory diagnostic errors, asymptomatic cases and even difficulties in differentiating COVID-19 from other diseases coexisted.

The data from this research revealed that the majority of those tested who tested negative, after careful analysis, were led to believe that they were false negatives, based on the type of inappropriate test, taking into account the date of onset of symptoms recommended by health authorities.

The main challenges for carrying out the initial diagnosis of COVID-19 include ideal biological material for testing, definition of biological marker to be detected, the type of methodology used and ideal time of infection for sample collection.<sup>10</sup> In the case of the RT-PCR kit, distributed by the Centers for Disease Control and Prevention (CDC) in China, it was designed to detect the nucleocapsid and ORF1ab, and infection is confirmed when both are amplified. However, it is possible that the results are inconsistent due to amplification of only one of the targets.

The testing strategy must consider the accuracy of tests for detecting antibodies, as the sensitivity and specificity of tests approved in Brazil vary between commercial kits from different manufacturers. Among the tests approved in the country, sensitivity is at low to moderate levels, which may imply difficulty in detecting infected individuals, especially in tests for the detection of anti-SARS-CoV-2 antibodies of the IgM class in initial

phase of infection.<sup>10</sup>

As of April 3, 2020, the Brazilian MoH had confirmed around 9,000 confirmed cases of COVID-19 (BRASIL, 2020). Detection of SARS-CoV-2 using real-time PCR test kits can be considered the gold standard for diagnosing COVID-19; however, this technique requires certified laboratories, more expensive equipment and trained technicians.<sup>11</sup> These characteristics were very far from the initial reality in the fight against COVID-19, being yet another in-depth demonstration of the statistics of a possible high number of false negatives.

Testing must respect the ideal interval recommended by manufacturers and the MoH; if this interval is not respected, these can be classified as negative when the patient is infected with the virus, generating false negatives.<sup>12,13</sup> Based on this assumption, taking into account the difficulties experienced at the beginning of the pandemic, it is expected that many negative tests will include positive patients. Adequate test management and effective patient clinical assessment, together with the correct completion of the notification instrument, were aspects considered relevant for the development of this study.

Analysis of these data suggests that the majority of discarded/negative tests may have been improperly analyzed, as they were performed outside the recommended time interval, based on the immunological window and manufacturer indications for each test. This indicates that the number of positive cases may be greater than the expected averages in the number of mobile cases reported by health units, not only related to analytical test errors, but also to epidemiological analysis, which includes the city's own demographics, which can make it difficult for patients to access health services, whether for geographic or economic and cultural reasons.

The limited scientific knowledge available, both for clarifying doubts and for training health professionals, had great significance, since, for hospital intervention and mass testing, a greater level of information would be necessary so that the results do not harm case management. Until patients meet the criteria according to pre-established protocols for reporting flu-like syndrome, they must be monitored as a suspected case of COVID-19.

Regarding the difficulty of clinical differentiation between the common cold and Influenza, these should be considered, with symptomatic cases and negative testing for COVID-19, suggesting the need for testing for other possible circulating viruses, considering that these patients classified as flu-like syndrome have not been diagnosed and confirmed for other syndromes.<sup>14</sup> Due to the totality of the sample and the epidemiological conditions of the pandemic, this percentage makes the chances of false negative results for COVID-19 questionable.

Furthermore, the high demand for tests, in some periods, caused their unavailability combined with the unpreparedness and lack of information on the part of professionals to face an emerging disease.<sup>14</sup> The immunochromatographic antibody test was the one that presented the best results in relation to the analytical phase of the test, as it was carried out in the appropriate period,

since approximately 6.3% of tests were carried out outside the recommended range, and this is probably due to the longer period available for them to be carried out.

Data analysis and presentation in this study favor the adoption of integrated public policies and measures, aiming to reduce prevalence rates. Furthermore, these data can be used to develop future studies in the area. Exposing these results to the scientific community and health professionals can better assist in choosing the ideal technique and management for each suspected case.

Furthermore, disseminating the study to the general population disseminates knowledge of the symptomatological differences between those affected and the relevance of the data acquired regarding the health promotion strategy. Although Brazil does not have a reference population for standardizing rates, it is noteworthy that incidence rates are directly influenced by the testing strategies adopted in the country and in each Federative Unit.<sup>15</sup>

To implement initiatives that guarantee continuous transmission control actions for tracking and testing suspected cases, it is necessary to use diagnostic methods that are easily accessible to professionals. As is the case with rapid virus antigen tests which, despite their low specificity and sensitivity, compared to the molecular technique, allow rapid detection of those infected, enabling early identification of cases, contact tracing and taking the necessary measures to greater control of the spread of the virus.<sup>16</sup>

Diagnostic approaches such as nucleic acid amplification tests (NAAT) such as RT-PCR are most widely used for the detection of SARS-CoV-2 infection, followed by rapid antigen tests<sup>17</sup>. However, they tend to give false positive and false negative results respectively. Therefore, it is important, in crisis situations, to have the availability and application of sensitive and specific testing measures for the virus being investigated.

As a limitation of this study, there is a high number of incomplete notifications, with approximately 14% of the data. The recording of dates, especially regarding the onset of symptoms and possible errors in filtering data when using analytical tools, is a reflection of the reality of overload in the healthcare system currently experienced. It is believed that these factors cause harm to the occurrence of worsening of the pathology and, consequently, facilitate the spread of the disease, which may have favored the pandemic. However, it is concluded that this is also a result of the critical analysis of data from this research.

It was also noted that the data coming from an early period of the pandemic, where everything was uncertain and testing measures were precarious, meant that notifications and results were less precise, requiring a deeper analysis to obtain promising results. However, this is also characterized by having a direct implication on public health policies, as it proves that testing measures, when not precise and specific, can lead to an increase in infected people through asymptomatic false negatives.

In short, it is observed that, in outbreak situations, variations in incidence arising from suboptimal testing

capacity must be differentiated from variations in real cases during monitoring. If the number of individuals reported as suspects is much higher than the testing capacity, this difference may lead to underdiagnosed cases.

It is important to highlight that inadequate testing implies unnecessary costs, with the acquisition of tests that do not provide specific results, in addition to influencing medical conduct, affecting the dynamics of infection containment, since case surveillance ends up being compromised.

Health policies must be based on testing systems and ensure their greater effectiveness, especially when dealing with infections that have an asymptomatic clinic, as this increases transmission, thus increasing the degree of epidemics and antigenic variations of the etiological agent, generating unwanted variants in a short period.

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## AUTHORS' CONTRIBUTIONS

**Anna Carolina Toledo da Cunha Pereira** contributed to project administration, bibliographic research and review. **Dénis Miguel Rodrigues de Oliveira** contributed to bibliographical research, writing the abstract, introduction, methodology, discussion, interpretation and description of results, preparation of tables, conclusions, review and statistics. **Deyseane Zacarias Freire de Sousa** contributed to bibliographic research, writing the abstract, discussion, interpretation and description of results, conclusions. **Gustavo Portela Ferreira** contributed to project administration, textual review of the abstract, methodology, results, conclusions, statistics. **Karliane de Araujo Lima** contributed to data availability, abstract writing, review and statistics. **Paloma Maria de Sousa Araujo** contributed to bibliographic research, introduction, methodology, discussion, interpretation and description of results, review and statistics. **Vanessa Poleana Silva** contributed to project administration, literature research, review and statistics.

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