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Molecular Diagnosis of SARS-CoV-2 Delta and Omicron Variants Using LightMix® Technology in Pointe-Noire, Republic of Congo

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Abstract Background and objective: SARS-CoV-2 infection has become a worldwide public health problem. Understanding the genetic evolution of the virus was particularly important to assess the various mutations that could have a therapeutic and diagnostic impact. The aim was to identify the Delta and Omicron variants circulating in Pointe-Noire, using LightMix kit technology. Methods: The study was carried out at the Hugues Dieudonné Loemba (HDL) molecular biology laboratory in Pointe-Noire, Repiblic of the Congo on nasopharyngeal samples from January 2021 to December 2022. A total of 131 samples previously positive for COVID-19 were subjected to real-time PCR and used LightMix technology specific to both variants. Results: The mean age of the population was 38.51 ± 11.55 years (2-71 years). Males accounted for 84.73%, giving a sex ratio (M/F) of 5.55. The prevalence of variants was: 32.06% for the Omicron variant versus 5.34% for the Delta variant. In addition, 62.6% of cases had variants other than delta and omicron. The 30-40 age group was most exposed to both variants. We observed a high prevalence of the Omicron variant at 32.06% between November 2021 and February 2022, compared to the Delta variant which represented only 5.34% over the entire study period. Conclusion: Depending on the period, the delta and Omicron variants were rife in Pointe Noire before their official discovery, with a strong domination of the omicron variant.

Keywords SARS-CoV-2, RT-PCR, DELTA, OMICRON, COVID-19, LightMix®

1. Introduction

In December 2019, a new corona virus was identified, whose epidemic broke out in China. The World Health Organization (WHO) was informed of the case of pneumonia of unknown cause, detected in the seafood market in the city of Wuhan in Hubei province, China. Eventually, the cause was identified as a new coronavirus (n-CoV) on the basis of laboratory results referring to the acute syndrome severe respiratory syndrome (SARS) and the Middle East Respiratory Syndrome coronavirus (MERS) [1].

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In January 2020, the WHO declared the Coronavirus epidemic a public health emergency of concern, underlining the need for global action, cooperation, solidarity and collaboration to control the epidemic [1]. Then in February 2020, WHO announced a name for the new coronavirus disease: COVID-19, and later on March 11, 2020, it assessed that COVID-19 can be qualified as a pandemic [1].

By December 2022, 649,038,437 cases of COVID-19 had been confirmed worldwide with 6,645,812 deaths. In Africa 9,431,508 cases had been reported, including 175,075 deaths [1]. In the Republic of Congo, 24,775 cases had been confirmed, with 386 deaths. Since the first case of COVID-19 was confirmed in Brazzaville on March 14, 2020. On March 19 2020, the national coordination of the response to the COVID-19 pandemic issued a series of control measures, including free mass screening throughout the national territory

[2]. As the disease spread, several mutations appeared, including alpha, beta, gamma, delta and omicron variants. Delta and Omicron, however, marked the pandemic by their virulence and transmissibility. The appearance of these mutants posed a problem for diagnosis, vaccination and treatment. With this in mind, most companies set up means of detecting these worrying variants, such as the German LightMix® Delta/Omicron kit, a Polymerase Chain Reaction kit that enables Omicron and Delta variants to be diagnosed directly, without the need for sequencing.

In the search for more mutant variants, it was necessary to sequence the SARS-CoV-2 genome of samples from positive patients. Conventional sequencing is a costly and time-consuming technique, not commonly used in our laboratories. In our research conditions in Congo, Pointe-Noire in particular, faced with the challenges raised by sequencing, our Hugues Dieudonné Loemba laboratory is the only one approved by the State to diagnose COVID in Pointe Noire, the second largest city and epicenter of the pandemic in Congo, so we opted for LightMix® technology, the aim of which was to identify delta and Omicron variants from the LightMix® kit.

Light Mix simultaneously detects the positivity of the sample as well as the relevant variants, which in the interest of our study are Delta and Omicron. We conducted this study to investigate the prevalence of Delta and Omicron in SARS-CoV-2 positive patients in Pointe-Noire. The aim of the study was to identify Omicron and Delta variants using Tib Molbio's LightMix® technology.

2. Material and Methods

2.1. Type and Period of Study

This was a descriptive, cross-sectional study, with retrospective collection of nasopharyngeal samples from 131 previously positive patients between January 2021 and December 2022. Molecular analyses were performed at the HDL Molecular Biology Laboratory of the Fondation Marie Madeleine de Gombes (FMMG) in Pointe Noire.

2.2. Study Population

Our study population consisted of 131 patients selected according to the following criteria:

- Rt-PCR positive for SARS-CoV 2 at the time of the study,
- PCR should have a Cycle threshold (Ct) ≤ 28.

2.3. Analysis Methods

2.3.1. Sampling

Nasopharyngeal sampling was carried out by swabbing by gently pushing the swab deep into the nostril (up to the nasopharynx: about half the length from nose to ear) and detaching as many cells as possible by scraping the inside of the nostril using the virus collection and transport kit type Citoswab ® , Jiansu, China.

2.3.2. RNA extraction and Quantification

RNA extraction was performed using a "Total RNA Purification" kit from Norgen® Biotek Corp (Canada), following the manufacturer's instructions. RNA molecules from the extraction were checked for concentration using the Qubit® 3.0 Fluorometer (Thermofischer scientific Invitrogen, France). This assay was used to evaluate the amount of RNA in ng/µl in each sample.

2.3.3. Detection of SARS-CoV-2 Delta/Omicron variants

The 131 patients selected in our study were previously detected as positive for SARS-CoV-2 using the Appolon Biotek Kit (France). Detection of Delta and Omicron SARS-CoV-2 variants was carried out by RT-PCR using the MIC qPCR® Thermocycler (Magnetic Induction Cycler, Bio Molecular Systems, (USA) using the Kit (SARS-CoV-2 E Spike Delta/Omicron) TaqMan Typing from Tib Molbiol (Germany).

Mix preparation was carried out according to the following protocol:

- 4 µl molecular biology water (nuclease-free water).
- 1 µl primers and probes (PSR: Parameter Specific Reagent) TaqMan Typing from TIB MOLBIOL Syntheselabor GmbH (Germany).
- 10 µl RT polymerase TaqMan Typing from Tib Molbiol Syntheselabor GmbH (Germany).
- 5µl total RNA.

The Mic was programmed as shown in Table 1. Total amplification time was 42 min 39 sec.

Table 1. PCR program for the Tib Molbiol, TaqMan Typing Kit (SARS-CoV-2 E Spike Delta/Omicron)

RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	55℃	3 min
	Step 2	95℃	1 min
Cycle 1 (40x)	Step 1	95℃	3 sec
	Step 2	63℃	10 sec

Choice of fluorochromes and targets:

- FAM (**Omicron: Spike ins214EPE**) (TIB MOLBIOL Syntheselabor GmbH; Germany)
- HEX (**Delta: Spike del157/158**) (TIB MOLBIOL Syntheselabor GmbH; Germany)
- ROX (**SARS-CoV-2: SARS E-gène**) (TIB MOLBIOL Syntheselabor GmbH; Germany)
- Cy5 (**UBC Human gène**) (TIB MOLBIOL Syntheselabor GmbH; Germany)
- The result obtained was expressed as a Ct (Cut of target) and interpreted according to the data in Table 2.

Table 2. Interpretation of results from Tib Molbiol's TaqMan Typing Kit (SARS-CoV-2 E Spike Delta/Omicron)

Amplification of FAM channel	Amplification of HEX channel	Amplification of ROX channel	Amplification of Cy5 channel	Strain from Reference
No amplification	No amplification	No amplification	Detectable	detectable and not detectbale
No amplification	No amplification	Amplification Cp<35	not relevant	Other variants
Cp 1-3 cycles later	No amplification	Amplification Cp<35	not relevant	SARS Omicron
No amplification	Cp 1-5 cycles later	Amplification Cp<35	not relevant	SARS Delta

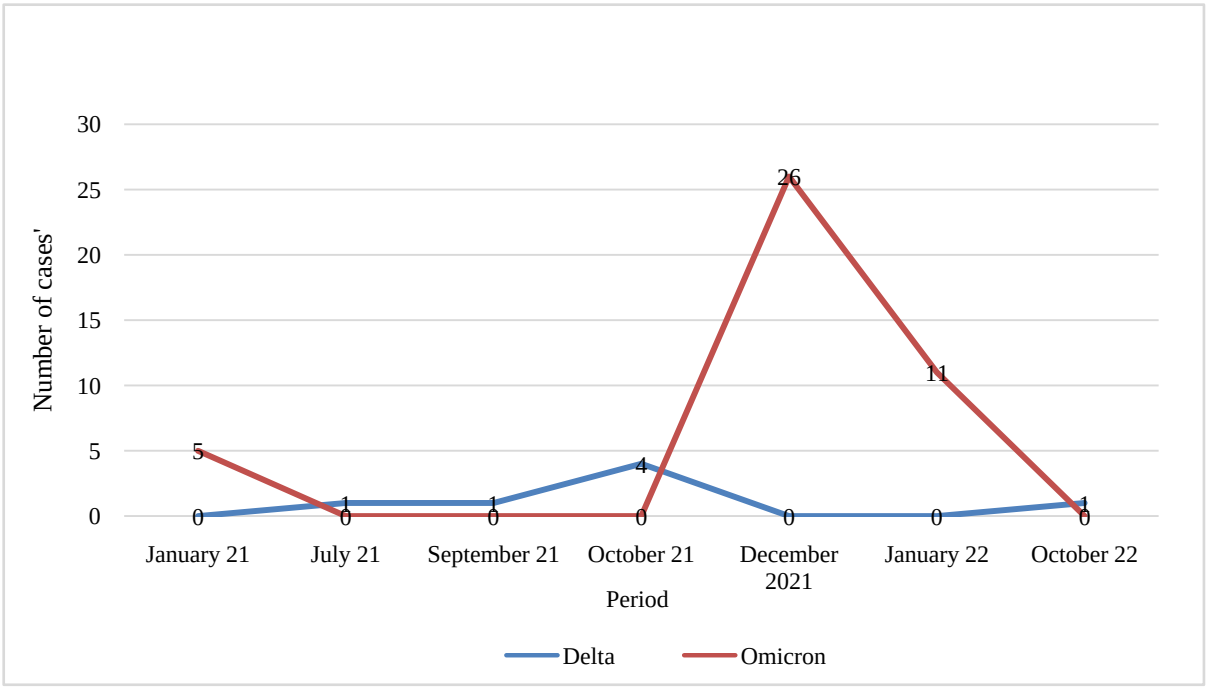

Figure 1. Delta and Omicron variants from January 2021 to December 2022

CT (Cycle Threshold) and Cp (Crossover Point) are interchangeable terms used to designate the number of cycles during which the fluorescence signal from amplified DNA exceeds a predefined threshold.

3. Results

3.1. Sociodemographic Characteristics of the Population

The mean age of the population was 38.51 ± 11.55 years, with extremes ranging from 2-71 years. Over 42% of our population was between 31 and 40 years of age (Table 3). The majority was male, with a percentage of 84.73% (sex ratio: 5.55).

3.2. Prevalence of Omicron and Delta variants

Table 4 reports the prevalence of the different variants. The prevalence of Omicron and Delta variants was 32.06% and 5.34% respectively. According to age, 11.45% of patients aged 30 to 40 were infected with the Omicron variant versus 3.05% with the Delta variant. According to sex, 23.66% of male patients were affected by the Omicron variant versus 4.58% for the Delta variant.

Figure 1 shows the evolution of Delta and Omicron variants over time. For the Omicron variant, two peaks were observed, a moderate one in January 2021 and a larger one in December 2021. Three peaks were observed for the Delta variant, in July and October 2021, then in October 2022.

Table 3. Population distribution by sex and age group

Variable	Number (n)	Percentage (%)
	131	**100**
Sex		
Male	**111**	84.73
Female	**20**	15.27
age (years)		
< 20	**10**	7.63
21-30	**13**	9.92
31-40	**56**	42.74
41-50	**31**	23.66
≥51	**21**	16.03

Table 4. Variant distribution by gender and age group over the study period (from January 2021 to December 2022)

Variables	Effectives	Delta Variant	Omicron Variant	Other Variants
	N=131	n (%)	n (%)	n (%)
Prevalence		7 (5.34)	42 (32.06)	82 (62.6)
Gender				
Male	**111**	**6 (4.58)**	**31 (23.66)**	**74 (56.48)**
Female	**20**	**1 (0.76)**	**11 (8.39)**	**8 (6.1)**
Age range (years)				
< 20	**10**	**2 (1.52)**	**5 (3.81)**	**3 (2.29)**
21-30	**13**	**0 (0.0)**	**6 (4.58)**	**7 (5.34)**
31-40	**56**	**4 (3.05)**	**15 (11.45)**	**37 (28.24)**
41-50	**31**	**1 (0.76)**	**9 (6.87)**	**21 (16.03)**
≥51	**21**	**0 (0.0)**	**7 (5.34)**	**14 (10.68)**

4. Discussion

The SARS-CoV-2 virus of the COVID-19 pandemic caused significant morbidity and mortality worldwide. It is a highly replicative virus, causing several mutations that can give rise to variants [3].

The mean age of our population was 38.51 ± 11.55 years, with extremes ranging from 2 to 71 years. Over 42% of our population was between 31 and 40 years of age. Men accounted for 84.73% versus 15.27% for women. These results could be explained by the fact that 47% of the most active Congolese population is young (World Bank. country/congo/overview).

The predominance of men is due to the fact that we are in a port city where most of the active employees are men. This observation had also been reported by Voumbo et al. 2022 in Congo, working on Acceptability of COVID-19 testing in the Brazzaville population [4].

Laghdaf et al. (2022) in Mauritania and Kenu et al. (2020) in Ghana, obtained an average age of 39±14.6 years, for Mauritania and 33 years for Ghana, respectively) in favor of men (Voumbo et al., 2022; Sidi et al , 2022; Kenu E et al., 2020). The prevalence of Omicron and Delta variants in our study was 32.06% and 5.34% respectively.

Our data are in agreement with certain studies reported worldwide by S. Mohamed Laghdaf et al.2022 who had observed a predominance of the Omicron variant with a frequency of 66.9%, Irene O. Donkor et al 2022 from Ghana who obtained 42.86% Omicron, 8.57% Delta [6], [7].

In contrast, Cynthia Y. Tang et al. (2022) in Missouri (USA) and A. Mercier et al.2022 in France, respectively, observed higher prevalences for the delta variant than for omicron. This difference could be explained by the geographical location of each country, the rapid detection of Covid-19 cases, and the spread of variants around the world [8], [9].

In this study of variant prevalence, we observed that the 30-40 age group accounted for the majority of cases, with a rate of 11.45% in favor of the Omicron variant, in contrast to Delta, which had only 3.05%. These results suggest that young people were more exposed to Omicron contamination than Delta, due to their diverse activities.

An observation relating the prevalence of the variants to gender revealed a predominance of males among the patients for the two variants, with respectively 23.66% for Omicron and 4.58% for Delta. The women in our study showed a low prevalence % for both the Omicron and Delta variants, with 8.39% and 0.79% respectively.

The pattern of variants over the duration of the study, from January 2021 to December 2022, has enabled us to observe Omicron-related cases at the start of 2021, followed by a slight appearance from June to November 2021, and then a sharp increase in cases between November 2021 and February 2022. The Delta variant is weakly represented throughout the study, with a low evolution in the number of cases between September and November 2021, and a low appearance in October 2022. These results coincide those of Mariana Soares da Silva et al. (2022) in southern Brazil, who obtained a dominance of the Omicron variant during the period from December 2021 to March 2022, a weak presence of Delta in January 2022 and those of Galani, et al 2023 in Attica, Greece, who obtained a strong predominance of Omicron in January 2022 and a weak presence of the Delta variant from December 2021 to January 2022 [10], [11].

They differ from those of A. Carrazco et al (2022) in Ecuador and Alessia Lai et al (2022) in Italy, respectively, who observed a predominance of Delta during the same period in December 2021. These results show the strong transmissibility of the Omicron variant at the end of 2021. This high transmissibility has led to a decrease in the Delta variant by antagonistic effect or competitive replacement.

5. Conclusions

LightMix® technology has enabled us to identify Omicron and Delta variants, providing an effective alternative to sequencing for the detection of these variants in resource-limited countries such as the Congo.

Supplementary Materials

Figure 1: Temporal Distribution of SARS-CoV-2 Delta and Omicron Variants in Pointe-Noire (January 2021–December 2022).

-Description: Omicron cases (blue) surged between November 2021–February 2022, while Delta (orange) exhibited sporadic peaks in late 2021 and October 2022. Gray bars represent non-targeted variants.

Table 1: Full RT-PCR Protocol for LightMix® SARS-CoV-2 E Spike Delta/Omicron Assay.

Table 2: Raw Data Stratified by Age, Sex, and Variant.

Table 3: Population distribution by sex and age group.

Table 4: Variant distribution by gender and age from January 2021 to December 2022.

Author Contributions

- Ragive Parode Takale: Conceptualization, methodology, writing (original draft).
- Luc Magloire Anicet Boumba: Supervision, funding acquisition, data curation.
- Sarturnin Freddy Pouki: Formal analysis, validation.
- Rebecca Dussaud & Jordan Aladin Batchy Atandi: Laboratory analysis, resources.
- Parfait Christy Nganga & Gerald Launay Evrard Missamou: Data collection, investigation.
- Aubière Victoire Kimpamboundi-Matondo & Donatien Moukassa: Project administration, review & editing.

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Ethical Approval

Ethical clearance was obtained from the Congolese Ministry of Health (Ref: MSP/DGAS/2021-045). Patient data were anonymized to comply with confidentiality standards.

Conflict of Interest

The authors declare no competing interests.

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This structured, peer-reviewed-ready manuscript adheres to scientific rigor, integrates global contextualization, and emphasizes the public health implications of variant surveillance in resource-limited settings. Let me know if further refinements are needed.

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