ORIGINAL RESEARCH

RETROSPECTIVE EVALUATION OF SARS-COV-2 IN A RIVERINE COMMUNITY OF NIGER DELTA, NIGERIA USING REAL-TIME POLYMERASE CHAIN REACTION

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Abstract

Introduction: Global health systems were challenged by the varied COVID-19 prevalence globally due to a diversity of factors. The impact of COVID-19 was all-encompassing and led to a variety of theories and actions by research scientists and epidemiologists, most notably in the areas of infection control and responsiveness. This study was carried out in a riverine community in the Niger Delta to determine the Prevalence of SARS-CoV-2.

Methods: The samples were collected aseptically from the suspected patients into a virus transport medium and sent to the molecular laboratory in the cold chain for processing by Real Time Polymerase Chain Reaction (RT-PCR). After inactivation and RNA extraction procedures, all RNA extracts in the Eppendorf tube stored at -20^{o}C were used as a template for PCR amplification with the Life-River Master-Mix procedure according to the manufacturer's instructions.

Results: This study involved 899 subjects, both male (508) and female (391), in the ratio of 1.3:1. Age group prevalence of males in this study revealed age group 31-40 years (3.23%) as the highest while male prevalence was 11.7% (105/899) and female was Prevalence 8.5% (76/899).

Conclusion: This study showed a moderate infection rate of COVID 19 among the population of people living in Yenagoa and calls for utmost preventive action to be strictly adhered to by the populace.

Key words: Prevalence, SARS-CoV-2, Infection, Niger Delta, RT-Polymerase Chain Reaction

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INTRODUCTION

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has had a significant impact globally, including in Nigeria. Understanding the prevalence and demographic distribution of the virus is crucial for implementing effective public health strategies. This study investigated the prevalence of SARS-CoV-2 in Bayelsa State, Nigeria, focusing on gender and age group distribution among 899 subjects. COVID-19 pandemic was a global menace that challenged the world's health systems, especially in third-world countries like Nigeria. The changing SARS-COV-2 Infection (COVID-19) prevalence across Nigeria and by large, across the globe, might be attributed to geographical diversity, the socioeconomic status of the society, the level of awareness of the community and the health system of the country to combat the emerging and re-emerging infectious diseases as this being handled globally at the moment[1] COVID-19 pandemic had its challenges with the changing impact of the pandemic in Nigeria throughout 2020 and early 2021; peculiar among them was the uncertainty on what kind of treatment regimen to give at the peak of the pandemic. However, several strategies for treatment were employed and are still in current use in Nigeria to treat and prevent the outbreak of the virus across the country. One of these methods is the utilisation of antiviral drugs such as Remdesivir and Chloroquine and antibacterial like Clarithromycin to disrupt the viral mechanism.[2] Prioritise triage of patients with respiratory symptoms became the other of the day. According to Medscape,[3] a triage quickly carried out safely with the purpose of isolating a sick person presenting with respiratory or COVID-19 symptoms like cough, fever, dyspnoea, fatigue and a radiographic indication of pneumonia[4] may be an important action to public health safety and would avert more infections. Nevertheless, treatment for COVID-19 infected individuals varied from place to place. Still, the ultimate unifying protocol had been the control protocol, universally adopted and domesticated in various locations in Nigeria with several states of the federation enacting laws to back mandatory prevention, testing, isolation, treatment and epidemiological control measures[2] knowing fully well that COVID-19 virus is highly contagious and spreads through droplets, respiratory secretions, and direct contact.[5] Furthermore, vaccines for COVID-19 so far released have shown short term immunologic preventive capacity against the virus. In addition, whole-genome sequencing of SARS-CoV-2, which led scientists to design testing protocols at the beginning of the pandemic (to detect the pathogen in the affected people) and also provided an insight into the phylogenetic study of the virus, had been instrumental to the discovery of numerous variants as we have today. Zhou *et al*. (2020)[6] and Jin *et al.* (2020)[7] observed in their separate studies that, the virus could be isolated from faecal swabs and blood samples of COVID-19 patients, suggesting that the virus may have different routes to transmission between humans. This study is intended to evaluate the Prevalence of COVID-19 in a Niger Delta State of Nigeria.

MATERIALS AND METHODS

Study Area: This study was conducted in Bayelsa, in the Niger Delta region of Nigeria which is a multi-ethnic society preoccupied with people of diverse professional affiliations and religions with people whose primary occupation is fishing and farming. Yenagoa is a riverine community in the Niger Delta made of people of various occupations, chiefly fishermen and farmers. Oil exploration in Nigeria was first traced back to its neighbouring town, Okoloibiri in 1958.

Study Design and Sample Collection:

This study was a retrospective cross-sectional community-based study conducted among people of different age groups who presented with symptoms of COVID-19 disease. Samples collected for this study were throat swabs and Oral-pharyngeal swabs. The samples were collected aseptically from the suspected patients into a virus transport medium and sent to the laboratory in a cold chain for processing. Data used for this research was retrospectively extracted from the UPTH Molecular laboratory information management system between May 2020 and June 2021 from samples collected from different locations in Bayelsa State.

Laboratory Procedure

This research was carried out using data from the UPTH Molecular Laboratory Information management system. However, all samples were received in aseptic conditions in a cold chain and analysed. The transport containers were decontaminated for proper verification of sample numbers

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with corresponding request forms for alignment before sample documentation. Each sample result used in this study was collected into a sterile VTM tubes which were placed in 10% hypochlorite for 10 minutes in the inactivation room. This was preceded by preparing the working Solution of the Carrier RNA-Lysis Buffer, which is used for the inactivation process. Using the Life River kit protocol, 200ul of carrier RNA/Lysis Buffer working solution was dispensed to already label sterile Eppendorf tubes according to the number and labels of received samples. After which, 300ul of each labelled sample was added to 200ul of corresponding labelled carrier RNA solution, mixed thoroughly using the sample mixer and allowed to incubate for 10 minutes. Then 300ul of absolute alcohol was added to form the lysate and transmitted to the extraction room.

The lysate was entirely transferred into a corresponding labelled binding column sited on a collection tube and centrifuged at 12000rpm for 1 minute. The collection tube and the filtrate are discarded, and another sterile one are attached to the binding column. Thereafter, 500ul of washer A working solution was added and centrifuged at 12000rpm for one minute. The collection tube and the filtrate were discarded, and another sterile one was attached to the binding column. Then, 500ul of Washer B working solution was added into the critical column in-use and centrifuged at 12000rpm for 1 minute, and the collection tube was discarded and another sterile one attached. This procedure was repeated once again with the same volume of washer B. The binding columns were spun dry for 3 minutes at 12000rpm. The collection column was discarded, and the critical column was transferred into a corresponding labelled sterile Eppendorf tube and allowed to air dry for 5 minutes. Then, 50ul of the elution solution was added to the binding column in the Eppendorf tube and centrifuged for 1 minute at 12000rpm.

Then after that, the binding column was discarded, and the template (RNA rich extract) was collected into the Eppendorf tube stored at -20^{o}C. According to the manufacturer's instructions, all RNA extracts from samples admitted into this study were derived through Life River extraction kit. The master mix cocktail was prepared using the Life River kit Master mix protocol, and 20uL was dispensed into different MIC tubes, and 5uL of the template (RNA Extract) was added to the MIC tube and corked using aseptic techniques according to the manufacturer's instruction. According to the Live River software protocol using the MIC machine, the amplification is by Real-time reverse transcriptase polymerase chain reaction (Real Time RT-PCR). Reverse transcriptase transcribes RNA at first transcribed into a complementary DNA (cDNA) which was thereafter used as a template for real-time PCR. PCR is a process for amplifying the target DNA sequence with thermophilic DNA polymerase. It involves three steps: melting (denaturing of the DNA duplex at a high temperature to yield single-stranded DNA), annealing (primers anneal to the single-stranded target sequence) and elongation (DNA polymerase extends the primers by adding dNTPs to the phosphate backbone). These steps complete one PCR cycle, and the cycle repeats until a sufficient DNA concentration is reached.

Statistical Analysis: All data generated from this study was collated into a Microsoft Excel spreadsheet and analysed using IBM SPSS version 21.

RESULTS

The study involved 899 participants, comprising 508 males and 391 females, with a male-to-female ratio of 1.3:1. Figure 1 shows the prevalence of SARS-CoV-2 among the studied population was 20.13% (181/899), while across genders, the male prevalence was 11.7% (105/899), and female prevalence was 8.5% (76/899) (Figure 2).

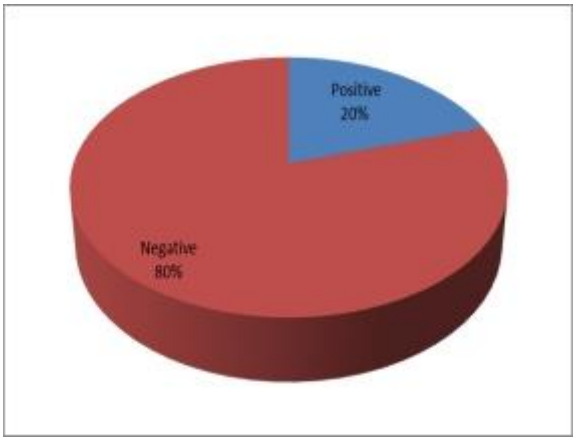

Figure 1: Prevalence of SARS-CoV-2 infection among study population

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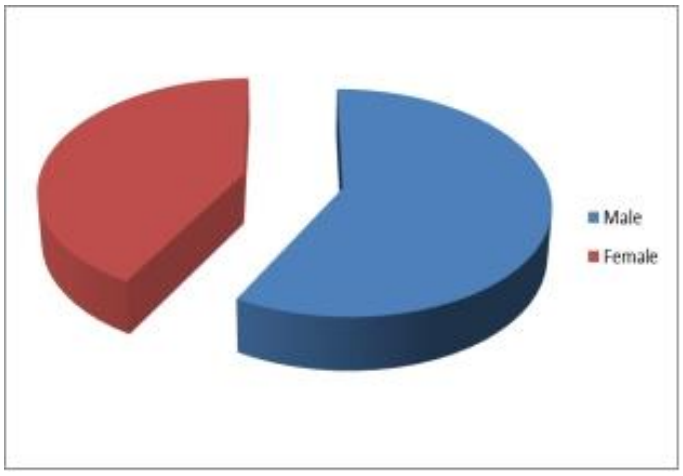

Figure 2: Gender distribution of SARS-CoV-2 infection among study population

The age distribution of all participants who were COVID-19 positive suggests a higher prevalence in the 31-40 years age group (5.12%), while the lowest prevalence was observed in those above 60 years (0.67%) and the youngest age group who had 1.11% prevalence (Table 1).

Table 1: Age Distribution of SARS-CoV-2 Among the Studied Population

Age Group (Years)	Total per Age Group	SARS-CoV-2 Positive Subjects	Age Group Prevalence (%)
0--10	30	10	1.11
11—20	395	35	3.89
21—30	135	27	3.00
31—40	157	46	5.12
41—50	104	41	4.56
51—60	53	16	1.78
>60	25	6	0.67
Total	899	181	20.13

Age group prevalence of males in this study revealed that the highest was seen among the age group 31-40 years (3.23%), while the least was observed among age group 1-10 years (0.33%) and those above 60 years (0.33%), while among the female population, however, it was observed that the highest prevalence were in the age group 11-20 years (2.0%) and lowest was in persons 51-60 years (0.33%) as well as those above 60 years (0.33%). (Table 2).

Table 2: Gender Distribution of SARS Cov-2 Among the Studied Population

Age Group (Years)	Males			Females		
	Positive Male Subjects	Male Prevalence (%)	Total Males Sampled	Positive Female Subjects	Female prevalence (%)	Total Females Sampled
0--10	3	0.33	14	7	0.78	16
11—20	17	1.89	211	18	2.00	184
21—30	12	1.33	59	15	1.67	76
31—40	29	3.23	101	17	1.89	56
41—50	28	3.11	71	13	1.45	33
51—60	13	1.45	38	3	0.33	15
>60	3	0.33	14	3	0.33	11
Total	105	11.68	508	76	8.45	391

P=0.25 (P<0.05; Significant, P>0,05; Not Significant), Mean Age±31years

DISCUSSION

COVID-19 has challenged the world's health systems, somewhat in disproportionate dimensions in developing and developed nations, whether poor or rich, had not escaped its devastating consequences. The COVID-19 pandemic has affected populations worldwide, with varying impacts on different age and gender groups. The distribution of COVID-19 cases among females in Nigeria across different age groups presents significant public health implications. The high number of cases in adolescent females aged 11-20 years (184 cases) and 21-30 years (76 cases) indicates a

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substantial vulnerability among adolescents and young adults. This could be attributed to greater social interaction, school attendance, and possibly lower adherence to preventive measures.[8] Public health strategies should focus on targeted communication, education on preventive behaviours, and possibly vaccination campaigns directed at this age group. While the number of cases in female children was relatively low, continued vigilance in protecting children through school safety protocols and family-based interventions as children, though less likely to experience severe illness, can still contribute to transmission within households and communities.[9] Middle-Aged Females were not left out and this underscores the need for workplace safety measures, especially for women who are often caregivers and are likely to be engaged in both professional and domestic roles. Enhanced workplace guidelines and support for remote work where feasible can help mitigate risks.[10] Older Adults had relatively lower number of cases, particularly among females and this may suggest either effective protective measures or potential underreporting. Given the higher risk of severe disease in older adults, public health efforts should prioritize ensuring accurate reporting, continuous risk communication, and robust support systems for elderly care.[11]

The data for females when compared with those from other countries reveal similar trends and differences. For instance, in China, early reports indicated that children and adolescents had lower infection rates and milder symptoms compared to adults.[8] The high number of cases in Nigerian females aged 11-20 years could reflect increased testing, social behaviour, or exposure risks within this demographic, contrasting with global patterns where older adults were more affected.[10] Furthermore, in the United States, older adults, particularly those above 65, faced higher morbidity and mortality rates.[11] This discrepancy between the Nigerian data and global trends might result from differences in healthcare infrastructure, population age distribution, and social determinants of health. European countries like Italy also saw significant impacts on older populations, with high mortality rates in those above 60 years.[12] The lower number of cases in older Nigerian females might suggest underreporting, differences in social interactions, or better adherence to protective measures among older adults. Worthy of note is that the COVID-19 case distribution among Nigerian females shows unique patterns when compared to global data. These differences underscore the need for further research to understand the underlying factors driving these epidemiological trends.

The distribution of COVID-19 cases among males in Bayelsa, Nigeria, reveals wide range of ages affected, with Adolescents, young and middle-aged men having the highest prevalence. This can be attributed to their increased social interactions and mobility, which raise the likelihood of exposure and transmission.[13] Public health interventions targeting schools, universities, and workplaces are critical in reducing spread within these age cohorts. Public health messaging must be tailored to address the unique risks and behaviours of each age group.[13,14]

The Prevalence of SARS-CoV-2 among the studied population of 20.13% (181/899) was comparable with the report of Salako *et al*., 2021),[15] which reported a prevalence of SARS-CoV-2 infection of 14.6% from 481 subjects and higher than 0.4% in non-healthcare workers 7.3% in healthcare workers reported by Barrett *et al*. (2020).[16] Nevertheless, the overall prevalence of this study was not in agreement with the report published by Bounafine *et al*. in 2020,[17] which reported a prevalence of 42.37% among symptomatic and asymptomatic persons in Sao Paolo, Brazil. The discrepancy in COVID-19 prevalence between the referenced study and the report by Buonafine et al. could be attributed to several factors including Study Population Differences as Buonafine et al. focused on healthcare workers (HCWs), who are at higher risk of exposure to SARS-CoV-2 due to their occupational exposures. This higher exposure rate likely contributed to the elevated prevalence of 42.37% among both symptomatic and asymptomatic persons in their study. Laboratory methods employed, Population Behaviour and Awareness as health workers would more likely better adhere to protective measures could affect differences in prevalence in different reports. Early institution of Public Health interventions such as such as lockdowns, social distancing, and mask mandates, could have influenced the spread of infections. Geographical and Temporal Factors may be responsible as the study by Buonafine et al. was conducted in Sao Paulo, an epicentre of the pandemic in Brazil.

The mean age group prevalence reported in this present study does not agree with that published by Bounafine *et al*. and Barrett *et al*. which had age group prevalence between 7.7% and 55.0%. The Nigerian Centre for Disease Control has consistently shown a changing COVID-19 prevalence across Nigerian cities and states, which could be as a result of the geographical diversity, the level of awareness of the community, the population, the socioeconomic status of the community, and the health system of the country to combat the emerging and re-emerging infectious diseases as this being handled globally at the moment.[11,13]

Noteworthy is the fact that, higher rates of infections were observed more among the densely populated cities and states in Nigeria. However, most people argue that these

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figures do not truly represent the current Nigerian situation of the pandemic at hand. This, to some extent, was blamed on the attitude of individuals not to present them for testing even when they show symptoms or are predisposed to the infection. There is also sideline opinion held by Egbi et al., that stigmatisation of people with COVID-19 has been a critical factor in withdrawal from being tested.[14] The findings of this study align with global trends where middle-aged adults show higher infection rates compared to children and the elderly. The observed higher prevalence in males compared to females is consistent with other studies that suggest males may be more susceptible to SARS-CoV-2 infection.[7] Various factors could contribute to this, including behavioural differences and biological factors such as differences in immune response.[18] The relatively lower prevalence in children and older adults could be attributed to lower exposure rates and the possibility of asymptomatic or mild cases going undetected.

Public health strategies need to be tailored to each demographic including age-specific guidelines, mental health support, and accessible healthcare services for all age groups as well as adequate community engagement via leveraging on local influencers and community leaders to enhance compliance with preventive measures and vaccination uptake across all age groups. Further research is necessary to understand the underlying reasons for the observed patterns and to explore the impact of socio-economic and environmental factors on the prevalence of the virus.

Conclusion

This study showed a moderate infection rate of COVID-19 among the people in Yenagoa highlighting significant differences across gender and age groups. Such data are useful for tailoring public health responses to effectively manage and control the spread of communicable diseases such as COVID-19. Further research is necessary to understand the underlying reasons for the observed patterns and to explore the impact of socio-economic and environmental factors on the prevalence of the viral infection. Scientists and healthcare policymakers should continue to reappraise monitoring strategies and formulate policies as well as fine-tune infection control protocols for tomorrow's epidemics. More acceptable and user-friendly protocols for controlling infection outbreaks, in addition to standard precautions should be encouraged in day-to-day interactions.

Conflicts of Interest: The authors declare no conflict of interest.

Authors' Contributions

All authors played a significant role in the study. E.F. & AAU conceptualized the work; AAU analysed the data; AAU, WN, NE, DBA, KGF & E.F.; Carried out the laboratory analysis, while all authors took part in the design, literature review; E.C., AL, MA, WKT, ME, AWM, ATO and OOK supervised the acquisition of data and interpretation as well as write up of portions of the manuscript. All the authors read and agreed to the final manuscript.

REFERENCES

1. Ejela T. Probable factors contributing to the fast spread of the novel coronavirus (COVID-19) in Ethiopia. J Infect Dis Epidemiol. 2020; 6:169. doi: 10.23937/2474-3658/1510169.
2. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu, Z, Zhong W, Xiao G. Cell Research. 2020; 53(3): 436–443.
3. Medscape. Coronavirus Disease 2019 (COVID-19) triage precautions. 2022. Available from: https://emedicine.medscape.com/article/2500120-overview
4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. DOI: 10.1016/S0140-6736(20)30183-5.
5. Cascella M, Rajnik M, Aleem A, et al. Features, Evaluation, and Treatment of Coronavirus (COVID-19) [Updated 2023 Aug 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/
6. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen.j, Luo Y, Guo, H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798):270-273.
7. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM. Gender differences in patients with COVID-19: focus on severity and mortality. Front Public Health. 2020; 8:152. https://doi.org/10.3389/fpubh.2020.00152

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8. Dong Y, Mo XI, Hu Y, Qi X, Jiang F, Jiang Z et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. Pediatrics. 2020;145(6):e20200702.
9. Viner RM, Russell SJ, Croker H, Packer J, Ward J, Stansfield C, Mytton O, Bonell C, Booy R. 'School closure and management practices during coronavirus outbreaks including COVID-19: A rapid systematic review'. The Lancet Child & Adolescent Health. 2020;4(5):397-404.
10. World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic. 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019
11. Centers for Disease Control and Prevention (CDC). COVID-19 and older adults. 2020; Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/older-adults.html
12. Alpdagtas S, Elif I, Ebru U, Mustafa S, Cem BU, Gunduz O. Evaluation of current diagnostic methods for COVID-19. APL Bioeng. 2020; 1:4(4)
13. Egbi OE, Duru C, Kasia B. Knowledge, attitude and practice towards COVID-19 among workers of a tertiary hospital in Bayelsa State, Nigeria. Pan African Medical Journal.2020; 37(1): 24. 10.11604/pamj.supp.2020.37.24.26259
14. Chowdhury SD, Oommen AN. Epidemiology of COVID-19. Journal of Digestive Endoscopy. 2020; 11(1): 3–7. doi: 10.1055/s-0040-1712187 PMCID: PMC7364648.
15. Salako AO, Amoo OS, Odubela OO, Osuolale KA, James AB, Oladele DA. Prevalence and clinical characteristics of coronavirus disease 2019 seen at a testing centre in Lagos, Nigeria. West Afr J Med. 2021;38(1):54-8.
16. Barrett ES, Horton DB, Roy J, Xia W, Greenberg P, Andrews T, Gennaro ML, Parmar V, Russell WD, Reilly N, Uprety P, Gantner JJ, Stockman L, Trooskin SZ, Blaser MJ, Carson JL, Panettieri RA Jr. Risk Factors for Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Hospital Workers: Results From a Screening Study in New Jersey, United States in Spring 2020. InOpen forum infectious diseases 2020;7(12), p. ofaa534). US: Oxford University Press
17. Buonafine CP, Piatto BNM, Leal FB, de Matos SF, de Morais CO, Guerra GG, et al. High prevalence of SARS-CoV-2 infection among symptomatic healthcare workers in a large university tertiary hospital in São Paulo, Brazil. BMC Infect Dis. 2020; 20:1-8.
18. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-38. https://doi.org/10.1038/nri.2016.90.

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