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Clinical Profile and Selected Viral Genome Sequence Analysis of Sars-Cov-2 Infected Pediatric Patients in A Tertiary Military Hospital in Quezon City from January 2021 – July 2023: A Cross Sectional Study

Abstract

Background: SARS-CoV-2 displays distinct characteristics in terms of virulence and disease severity in pediatric population. Hence, patient's clinical profile and viral genome may contribute to patient's symptoms and disease severity

Objectives: To determine the clinical profile and to analyze selected viral genome sequence of pediatric patients infected with SARS-CoV-2 in a Tertiary Military Hospital in Quezon City from January 2021 – July 2023.

Methods: This is a single center cross sectional study determining the SARS-CoV infected pediatric patients' clinical profile consisting of age, sex, residence, comorbidities, presenting symptoms, hospitalization and disease severity. Selected samples were subjected to next generation sequencing to analyze the SARS-CoV-2 genome sequence. All data were recorded with utmost confidentiality.

Results & Conclusion: From January 2021 to July 2023, a total of 630 pediatric patients who got tested for SARS-CoV-2 RT-PCR were confirmed to have SARS-CoV-2. Two hundred thirty-nine (239) were included in this study. Among the study population, 61.51% were male, more than 60% were less than 4 years old and residing in Quezon City. In terms of clinical presentation, 78% had mild symptoms, 20% had radiologic findings of pneumonia, 40% presented with fever, 12% with diarrhea, and 8% with dyspnea. Ten percent (10%) of patients had concomitant seizure disorder. More than 70% were admitted to the hospital and all of the study subjects recovered from the illness. Only ten samples were subjected to next generation sequencing and all were Omicron variants. Majority of the SARS-CoV-2 virus belong to Clade 22B and 23E; and identified pangolin lineages were BA.5.2, BA.2.3.20, XBB.1.5, GJ.1.2 and FL.23.2.

Research Article

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Keywords: SARS-CoV-2, next generation sequencing, viral variants, clinical outcomes

Introduction

The novel coronavirus, SARS-CoV-2, has led to a highly contagious disease that cause a pandemic worldwide. The virus rapidly spreads and evolves as it courses from one continent to the other. It was also observed that several strains of this virus cause different clinical symptoms in different patients. Most notable are the alpha, beta, gamma, delta and omicron variants. Interestingly, infected pediatric population presented different clinical outcomes compared to adults with different strains of virus. [1] During the pandemic, researches on SARS-CoV-2 and their clinical impact then became an interesting field in epidemiology and infectious disease. There are several published researches on SARS-CoV-2 and their outcomes, likewise there are several published researches on the limited genome sequence analysis of SARS-CoV-2 in the Philippines. However, these researches are gathered in the adult popula-

tion. There is no published research paper yet dedicated to SARS-CoV-2 and their clinical symptoms on pediatric population in the Philippines. Hence, this research was created to investigate the clinical profile of SARS-CoV-2 infected pediatric population and even further look into their viral genome sequences.

Due to the rapid development of technology, specifically in the field of molecular biology, next generation sequencing (NGS) was developed in studying viral evolution. The use of NGS technology has become a widely accepted method for outbreak tracking and genomic epidemiology. [1] Detecting new mutations in SARS-CoV-2 allows the researchers to reconstruct unknown routes of infection and provide a molecular basis for SARS-CoV-2 diagnostic development, vaccine design and drug discovery. [2,3] Hence, viral genomic diversity become an important field in determining viral virulence, transmissibility and disease severity.

There were several researchers worldwide reporting the NGS of SARS-CoV-2 and correlating their clinical symptoms. In the Philippines, coding of SARS-CoV-2 genome was first initiated in August 30, 2020. [4] But due to its high cost and rarity, limited specimen was subjected to NGS. [4] In recent times, there were little researches on SARS-CoV-2 genomic sequence in the Philippine settings, [4] more so in the pediatric population. Thus, this research aims to determine the clinical profile of SARS-CoV-2 infected pediatric patients and analyze selected viral genome sequence in a tertiary military hospital in Quezon City. Determining this genome profile will be a vital data that will contribute in the study of SARS-CoV-2 viral evolution in pediatric population in the Philippines. Data in this research could be of a great value in future reference, especially in the field of epidemiology and vaccine development.

Objectives

General Objectives: To determine the clinical profile and to analyze the selected viral genome sequence of pediatric patients infected with SARS-CoV-2 in a Tertiary Military Hospital in Quezon City from January 2021 – July 2023.

Specific Objectives:

1. To determine the clinical profile of patients infected with SARS-CoV-2 in a military hospital in Quezon City from January 2021 – July 2023:
   a. Age
   b. Sex
   c. Place of Residence
   d. Comorbidities
   e. Presenting Symptoms
   f. Hospitalization
   g. Disease Severity
2. To analyze the SARS-CoV-2 genome sequence of selected pediatric population using next generation sequencing in a Tertiary Military Hospital in Quezon City from January 2021 – July 2023.

Ethical Consideration

Whole-genome sequencing was performed by collecting the oropharyngeal / nasopharyngeal samples that were collected in the context of routine diagnosis. No additional samples were collected for this study. Clinical profiles were retrieved from the medical files and anonymized before analysis. Ethical approval was obtained from the AFP Medical Center Research Ethics Committee.

Review of Related Literature

During the late December 2019, a novel coronavirus was detected in Wuhan, China. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has become a global concern taking millions of lives throughout the globe. [5] The novel virus is capable of rapid transmission through the respiratory epithelium that infects every population that varies from age, race and gender.

This virus is detected through Reverse Transcription Polymerase Chain Reaction (RT PCR) collected from samples of the oral and nasal area of patients. [5] But during the pandemic, the virus evolved, from subvariants that causes minimal symptoms to severe and critical outcomes. Disease morbidity and mortality differs from different variants, e.g. the delta variants were more virulent and capable of infecting the pediatric population. [6] Hence, the use of next generation sequencing (NGS), a technology used to investigate the genetic makeup of all living forms capable of sequencing billions of nucleic acid fragments simultaneously has been widely use in studying viral variants. [7] According to the Philippine Pediatric Society (PPS) most children and adolescents infected with SARS-CoV-2 present with mild to moderate symptoms and only a small percentage (0.9%) of patients develop severe and critical disease. [8] There are several theories formulated attempting

to explain the difference in severity and susceptibility of children compared to adults that includes reduced number of ACE 2 receptors and immature immune systems. [8] However, it was observed that weekly COVID-19 hospitalization rates in children and adolescents have varied throughout the pandemic. [9] Peaking initially in January 2021 (1.5 per 100,000 population), again in September 2021 with predominance of the Delta (B.1.617.2) variant (1.8 per 100,000 population), and then again in January 2022 with predominance of the Omicron variant (7.1 per 100,000 population). These differences in viral peak opened up a new door of possible different outcomes with different strains of viruses. [9] The viral genomic sequencing may pose a great potential in the field of epidemiology and medicine.

Infected pediatrics patient's clinical profile may greatly contribute to their outcome, this include age, sex, comorbidities and their initial presentation. [8] On the contrary, viral strains may not affect the outcome of most patients, but the COVID-19 variants lead to different outcomes manifesting a unique behavior. [9] This may indicate that SARS-CoV-2 viral genomic diversity could be a key in their viral virulence. Like other viruses, SARS-CoV-2 evolves over time. Most mutations in the SARS-CoV-2 genome have no impact on viral function. However, the following notable SARS-CoV-2 variants has different clinical outcomes. [10] **The Alpha (B.1.1.7 lineage)** was first identified in the United Kingdom in late 2020 and subsequently became the first globally dominant variant. Alpha was approximately 50 to 75 percent more transmissible than previously circulating strains and it was associated with greater disease severity. [10]

Another is the **Beta (B.1.351 lineage)**, also known as 20H/501Y.V2, was identified and predominated in South Africa in late 2020. [10] The main concern with Beta variant was immune evasion: convalescent and post-vaccination plasma did not neutralize viral constructs with Beta spike protein as well as those with wild-type spike protein. The **Gamma (P.1 lineage),** known as 20J/501Y.V3, was first identified in Japan in December 2020. Main concern with this variant is several mutations increasing concern about increased transmissibility and an impact on immunity. [10]

The **Delta (B.1.617.2 lineage)** lineage was first identified in India in December 2020 and been the most prevalent variant worldwide until emergence of the Omicron variant. Delta variant was more transmissible and was associated with a higher risk of severe disease, hospitalization and death.10

COVID 19 in Pediatric Population

During the Omicron dominant periods, weekly hospitalization rates were particularly high in infants younger than six months and in children age six months through four years, who were not eligible for vaccination until June 2022. [11] Among children age 5 to 11 years (who became eligible for vaccination in early November 2021), hospitalization rates were approximately twice as high in unvaccinated than in vaccinated children (19 versus 9 per 100,000) during the period of early Omicron predominance (December 19, 2021 to February 28, 2022). Higher proportions of unvaccinated than fully vaccinated adolescents had COVID-19 as the primary reason for hospitalization (70 versus 41 percent) and required intensive care (30 versus 16 percent). [11]

Several studies support that, COVID-19 disease severity may be related to the strain of SARS-CoV-2. In observational studies in children and adolescents, the rates of admission to the ICU and mechanical ventilation were lower with the Omicron than the Delta variant. [11]

In recent times, the cases of COVID 19 in our country are closely monitored including the variants that is currently dominant. The use of NGS technology has become a widely accepted method for outbreak tracking and genomic epidemiology.

Significance of the Study

This is one of the novel studies in the Philippines using next generation sequencing (NGS) to determine the SARS-CoV-2 genome sequence infecting pediatric patient in a tertiary military hospital. This paper may strengthen the importance of determining viral genome as a guide in the management and predicting outcomes of COVID-19 infected pediatric patients. This will greatly contribute to the contemporary studies worldwide on SARS-CoV-2 variants, the demographics they infect and their clinical symptoms. This can also serve as a literature in other future studies on SARS-CoV-2 genome sequencing and their clinical symptoms. Furthermore, this paper emphasized the importance of NGS and molecular biology in studying viral evolution as a part of diagnostic, therapeutic and vaccine development.

In the field of pediatrics, specifically during the COVID-19 pandemic, the viral NGS result was one of the vital data that contribute to the study of the virus. The different viral genome was correlated to the variant, and each variant display distinct characteristics and outcomes. With the aid of NGS, medical personnel become aware of the circulating virus variant and their behavior. This data helps in planning and formulation of strategies by our medical front-liners, especially during the virulent waves of delta and gamma, wherein healthcare providers become wary and vigilant as cases are expected to be moderate to severe. On the other hand, during the omicron wave, medical personnel expect a spike in number of infected patients but generally milder cases. With the advent of molecular technology, our understanding of infectious disease become wider and critical. We can identify the genetic make-up of pathogens and correlate it to their virulence. We can develop vaccines congruent to their specific molecular structures. The future of medical research maybe gearing towards understanding the genomic characteristics of the pathogen and its host, and this research is one of those papers pioneering this molecular approach.

Scope and Limitation

This is a single center cross sectional study assessing the clinical symptoms of SARS-CoV- 2 infected pediatric patients in a tertiary military hospital. This involves all pediatric patients seen in V Luna Medical Center from January 2021 – July 2023 that underwent SARS-CoV-2 RT PCR which revealed positive. In addition, selected samples were subjected to next generation sequencing for analysis. The major limitation of this study is the small number of samples, 10 sequences, hence testing association with other variables were not possible.

Methodology

Study Design

This is a single center cross sectional study determining SARS-CoV-2 genome sequence and patients' clinical profile in V Luna Medical Center from January 2021 to July 2023. Samples of throat and nasal swabs underwent SARS-CoV-2 RT-PCR and selected samples were subjected to next generation sequencing processed by Armed Forces Research Institute of Medical Sciences (AFRIMS).

Study Population and Setting

Sample population composed of pediatric patients positive for SARS-CoV-2 RT PCR tested in V Luna Medical Center from January 2021 to July 2023. Their demographic and clinical data were retrieved from medical files, including the general data, symptoms, and course of the disease. In addition, selected SARS-CoV-2 RT PCR positive samples with Cycle Threshold of <30 underwent next generation sequencing.

Sample Collection

Nasopharyngeal and oropharyngeal swabs were collected using Dacron-tipped swabs by clinical personnel. [12] Samples were collected from pediatric patients under investigation for COVID-19, patients seeing clinical care and showing signs of COVID-19-like illness. [12] Swabs were stabilized in various Universal Transport Media (Copan, CA, USA; Sansure Biotech, Hunan, China; and Sanli, Liuyang, China), temporarily stored at 4°C, transferred to freezers (−80 ± 20°C), and tested within 24 to 72 hours. [12]

SARS-CoV-2 Real-Time RT-PCR Testing

Nasopharyngeal and oropharyngeal swabs were collected by clinical staff and rRT-PCR testing was performed. The swabs were heat inactivated at 65°C for 10 minutes and viral RNA was extracted using the NATCH CS automated nucleic acid extraction machine (Sansure Biotech, Hunan, China) or manual RNA extraction kits: QIAmp viral RNA mini kit (Qiagen, Germantown, MD, USA), Sansure RNA one-step nucleic acid release reagent (Sansure, Biotech, Hunan, China), and GenAmplify RNA extraction kit (Manila HealthTek, Manila, Philippines) following manufacturers' instructions. [12] Briefly, specimens were thawed and an aliquot of 200 µL (Sansure) or 140 µL (QIAmp and GenAmplify) of each specimen was used for RNA extraction and eluted in 50 µL following manufacturer's instructions of each kit. [12] The following SARS-CoV-2 rRT- PCR kits were used following testing conditions and CT cutoff values indicated by each manufacturer: Sansure (Sansure Biotech, Hunan, China), 40 CT; BGI Genomics (Shenzhen, China), 37 CT; modified Berlin assay, 38 CT; and modified U.S. Centers for Disease Control and Prevention (CDC, USA) assay, 38 CT. The extracted nucleic acid was amplified using the following PCR machines: ABI 7500 (Applied Biosystems, CA, USA), QuantStudio 7 (Ap-

plied Biosystems, CA, USA), SLAN96P (Sansure Biotech, Hunan, China), and MA6000 (Sansure Biotech, Hunan, China). [12] Selected specimens also underwent GeneXpert PCR SARS-CoV-2 testing.

SARS-CoV-2 Genome Sequencing and Phylogenetic Analysis

SARS-CoV-2 genome from 10 selected positive specimens (CT < 30) were sequenced in the in-house molecular laboratory, using an iSeq (Illumina, USA), the results of which were reported and described [12] (Table 1).

Phylogenetic Analysis

Phylogenetic analysis was performed using sequences from this study and sequences retrieved from GenBank and GISAID databases on August 24, 2020. [12] The tree was generated using IQ-tree v.1.6.12 using the generalized time-reversible (GTR + F + I) model and 1,000 bootstrap replicates. Multiple sequence alignments were performed using “Multiple Alignment using Fast Fourier Transform” v7.407 with the default setting. [12] The phylogenetic tree was drawn with FigTree v1.4.4 and lineage was determined using Pangolin v.2.0.4 (github.com/cov- lineages/ pangolin), including the lineage and the clade of the 23 coding-complete genome sequences from this study. [12] The genome comparisons to the Wuhan-Hu-1 reference genome (GenBank accession number NC_045512.2) were visualized using BRIG v0.95. [12]

Conceptual Framework

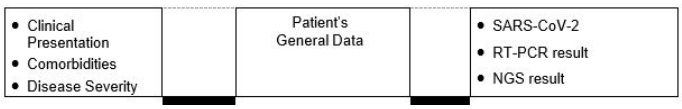

Figure 1: Shows the correlation of SARS-CoV-2, patient’s clinical profile and NGS data

Inclusion Criteria:

1. All pediatric patient 18 years old and below who consulted in VLMC form January 2021 – July 2023.
2. All pediatric patient who were positive with SARS-CoV-2 RT-PCR with and without symptoms.
3. All pediatric patient who were positive for SARS-CoV-2 RT-PCR and underwent next generation sequencing of SARS-CoV-2.

Exclusion Criteria:

1. Non-pediatric age group
2. Positive for SAR-CoV-2 RT-PCR done outside VLMC

Operational Definition of Terms

SARS-CoV-2. The novel coronavirus is the main variable of concern in this paper and includes, determination of its variants and their clinical outcomes in pediatric population.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR is the diagnostic test for SARS-CoV-2, all subjects in this paper were positive for SARS- CoV2 RT-PCR before proceeding to NGS.

Next Generation Sequencing (NGS)

Selected subjects in this paper underwent NGS in order to identify the SARS-CoV-2 genomic make-up and viral variants. Qualified samples are those with cycle threshold (CT) value of <30.

Data Collection

Epidemiological, clinical, and disease severity data were extracted from electronic medical records provided by V Luna Medical Center Record Section and Department of Pediatrics Database. All data of patient’s NGS was provided by the AFRIMS. All data records were matched with previously assigned sample IDs and used as anonymized dataset for downstream analyses. All data collected will be treated with utmost confidentiality.

Statistical Interpretation

Measures of central tendency/dispersion and proportion were used to describe quantitative and categorical variables.

Results

From January 2021 to July 2023, the Department of Pathology and Laboratory of V Luna Medical Center tested 103, 486 patients for SARS-CoV-2 RT-PCR, among which 5,945 (5.7%) were pediatric patients. Six hundred thirty (10%) pediatric patients were positive for SARS-CoV-2 and only 10 (1.5%) pediatric patients were subjected to next generation sequencing. (See Figure 2)

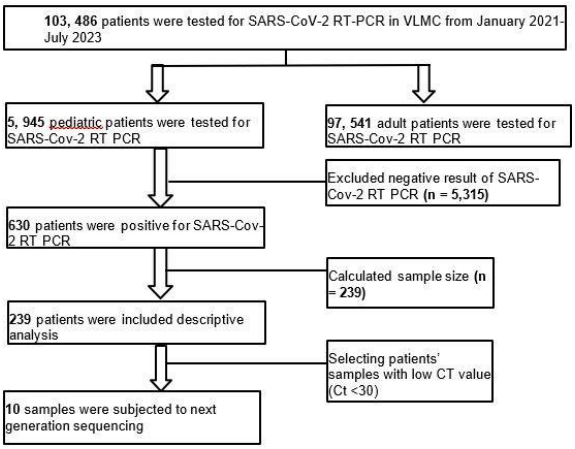

Figure 2: Flow chart of the selection of subjects in the study

A total of 239 patients were included in the study, and majority were male 61.51% (n = 147) vs female 38.49% (n = 92) as shown below (Table 1).

Gender	f	%
Male	147	61.51
Female	92	38.49

Table 1: Shown is the sex distribution of subjects included in the study.

Majority of the subjects were less than 4 years old. One to two years old comprised 27% (n=65), 1mo-11mos 22% (n=53) and 3-4 years old (n=29) as shown in Figure 3 below.

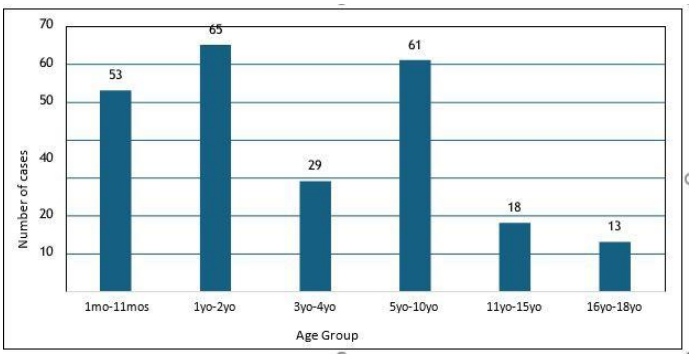

Figure 3: Age distribution of pediatric COVID 19 cases seen in VLMC

Most of the patients were from Quezon City 60.67% (n = 145), followed by Taguig 16.74% (n = 40) and Cavite 15.48% (n = 37) as shown below (Figure 4).

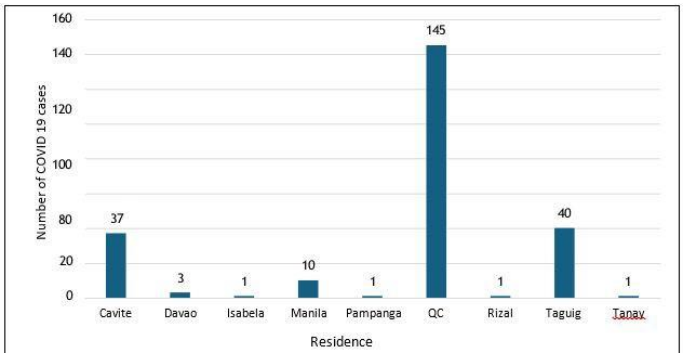

Figure 4: Residence distribution of pediatric COVID 19 cases seen in VLMC.

A quarter of the patients has comorbidities 38% (n = 59) while those with no comorbidities comprised 61% (n = 180) as shown below (Figure 5).

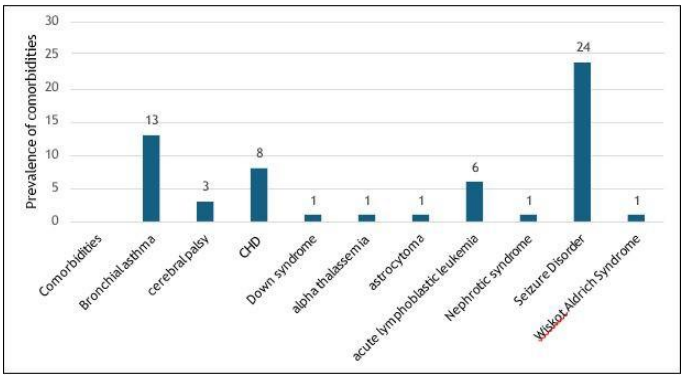

Figure 5: Prevalence of comorbidities among pediatric COVID 19 cases seen in

Most of the patients presented with fever 40% (n = 97) followed by diarrhea 12% (n = 30), cough 9% (n = 22) and dyspnea 8% (n = 21) as shown below (Figure 6).

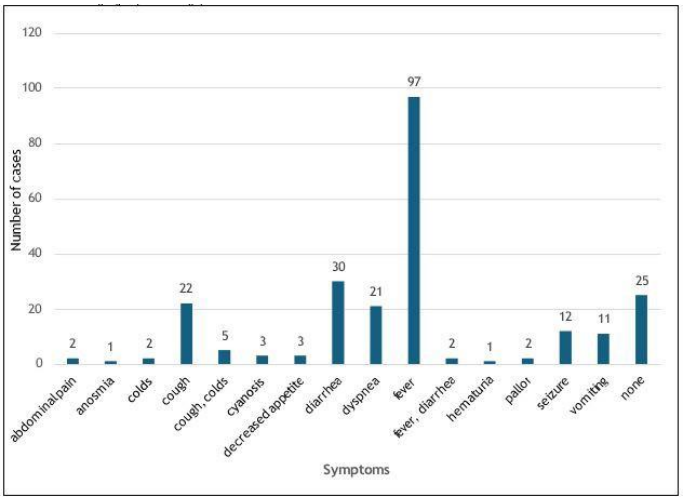

Figure 6: Distribution of symptoms of pediatric COVID 19 cases seen in VLMC.

Majority of the patients were admitted comprising 76% (n = 183) while 23% (n =56) were advised for home isolation as shown below (Table 2).

Hospitalization	f	%
Yes	183	76.57
No	56	23.43

Table 2: Shown is the number of subjects who were admitted in the hospital

Most of the patients present with mild symptoms, 78% (n = 187), followed by moderate symptoms 20% (n = 49) and 1% (n = 3) had severe symptoms as shown below (Table 3).

Disease Severity	f	%
Mild	187	78.24
Moderate	49	20.50
Severe	3	1.26

Table 3: Shown is the disease severity distribution of subjects included in the study.

More than half of the patients 61% (n = 148) had normal chest X-ray results, while 20% (n = 50) have pneumonia as shown below (Table 4).

Chest X-ray	f	%
Pneumonia	50	20.92
Cardiomegaly	3	1.26
Normal	148	61.92
Not done	38	15.90

Table 4: Shown is the chest x-ray results of subjects included in the study

Out of 239 SARS-CoV-2 positive patients, only ten (10) samples were subjected to NGS Illumina iSeq100TM. NGS was only done for specimen with low cycle threshold value (CT value < 30) that correlates to high viral load (equivalent to ~10,000–100,000 viral genome copies/mL) (Table 7). [7] Genome-wide characterization of SARS-CoV-2 isolates from 10 samples collected, generated 10 high-quality sequences using iSeq (Illumina, USA) as shown in Table 6. These ten samples revealed Ct value of less than 30: specimen (1) 27.11 – 28.27; specimen (2) 20.78 – 20.68; specimen (3) 15.50; specimen (4) 22.22 – 22.35; specimen (5) 21.68 – 22.10; specimen (6) 16.42 – 16.79; specimen (7) 17.90 – 19.70; specimen (8) 15.79 – 17.90; specimen (9) 27.75 – 34.42 and specimen (10) 19.93 – 20.75.

Sample Number	Obtained consensus sequences length (bp)a	Number of "N" (ambiguous base)	Percent reference coverageb	Median depth of coverage (x)	Nextclade Clade	Lineage (pangolin)	Cycle Threshold N1	Cycle Threshold N2	Cycle Threshold RP
1	28829	984	96.73	3557	22B (Omicron)	BA.5.2	27.11	28.27	28.98
2	27814	2024	94.11	1351	22B (Omicron)	BA.5.2	20.86	20.78	31.76
3	28738	1102	96.98	2708	22B (Omicron)	BA.5.2	15.50	14.99	26.29
4	29287	555	98.47	6576	21L (Omicron)	BA.2.3.20	22.35	22.22	26.17
5	28247	1596	95.59	1509	23A (Omicron)	XBB.1.5, VOI*	21.68	22.10	28.29
6	29354	489	98.23	5806	23D (Omicron)	FL.23.1	16.42	16.79	29.66
7	29360	484	98.22	4458	23E (Omicron)	GJ.1.2	19.70	17.90	27.3
8	23573	6263	80.90	979	23A (Omicron)	XBB.1.5, VOI*	15.78	17.94	30.78
9	29352	492	98.21	5818	23E (Omicron)	GJ.1.2	27.75	34.42	32.00
10	23759	6084	85.81	978	23E (Omicron)	GJ.1.2, VUM*	19.93	20.75	29.83
Ave.	27831.3	2,007.3	94.3						

Table 5: Ten SARS COV-2 genome sequence showing the sequences length, ambiguous base, median depth of cover, clades and lineage.

As shown in table 5, Nextclade classified all detected viral strain under Omicron variant. Figure 7 below lifted from Nextstrain.org shows the genomic diversity of SARS-CoV-2 identified in this study. [13] Majority of SARS-CoV-2 detected where from the lineages of 22B and 23E.

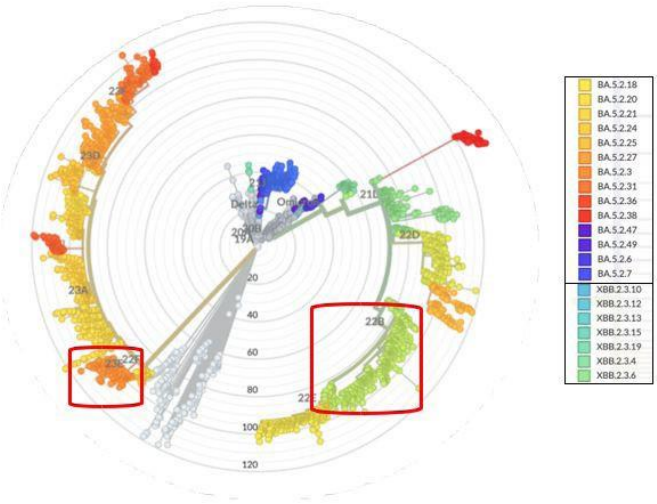

Figure 7: Shown is the complete phylogenetic tree of the novel SARS-CoV-2. [13] Highlighted is the major lineages 22B, 23E and their sub-lineages that were detected in this study. (Image lifted from Nextstarin.org)

Furthermore, pangolin lineages identified the following lineages: BA.5.2, BA.2.3.20, XBB.1.5, GJ.1.2 and FL.23.2 as shown in Table 5.

Specimens 1, 2 and 3 revealed Omicron BA.5.2 lineages, the Illumina run on these 3 clinical specimens obtained consensus sequences length of 28, 829 bp; 27, 814 bp and 28, 738 bp respectively. Mapping against Wuhan SARS-CoV-2 reference (NC_045512) revealed percent reference coverages of 96.73%, 94.11% and 96.98% respectively. These 3 specimens were collected from July to August 2022. Subjects were two males and one female, two were 2-year- old and one is 1-year-old, all were from Quezon City and they all came in due to fever. Two of the subjects were managed as COVID 19 Mild while the other one is managed as COVID 19 Moderate due to the findings of pneumonia on chest x-ray. None of the subjects has comorbidities and all have good clinical outcomes.

Specimens 5 and 8 revealed Omicron XBB.1.5 lineage, the Illumina run obtained consensus sequences length of 28, 247 bp and 23, 573 bp respectively. And mapping against Wuhan SARS-CoV-2 reference (NC_045512), the percent reference coverages revealed 95.59% and 80.90% respectively. These specimens were collected last April and May 2023. Subjects were two males, one is 1-year-old and one is 4-year-old, all were from Quezon City and they came in due to cough, colds and fever. One subject was managed as COVID 19 Mild while the other one is managed as COVID 19 Moderate due to the findings of pneumonia on chest x-ray. One subject has PTB and bronchial asthma, and all subjects have good clinical outcomes.

Specimen 7, 9 and 10 revealed Omicron GJ.1.2 lineage, the Illumina run obtained consensus sequences length of 29, 360 bp; 29, 352 bp and 23, 759 bp respectively. Further, mapping against Wuhan SARS-CoV-2 reference (NC_045512) showed percent reference coverages of 98.22%, 98.21% and 85.81% respectively. These 3 specimens were collected from March, May and June 2023. All subjects were female, one is 3-year-old, one is 2-year-old and one is 9-month-old; two is from Quezon City and one is from Tanay, and they all came in due to fever. One of the subjects was managed as COVID 19 Mild while the other two were managed as COVID 19 Moderate due to the findings of pneumonia on chest x-ray. One of the subjects has global developmental delay, hypothyroidism and nephrolithiasis but all have good clinical outcomes.

The other 2 specimens revealed unique lineages. Specimen 4 revealed Omicron lineage BA.2.3.20 with percent reference coverage of 98.47%. The specimen was extracted from a 3-year- old, male, from Quezon City with bronchial asthma. He was managed as COVID 19 Moderate due to pneumonia on chest x-ray and was discharged with no complications. On the other hand, the specimen 6 revealed Omicron lineage FL.23.1 with percent reference coverage of 98.23%. The specimen was taken from a 4-year-old, male from Quezon City. He was managed as COVID 19 Mild and was discharged with no complications.

In terms of demographics and clinical profile, the sex distribution of these 10 patients with NGS was 60% (n = 6) males, and 40% (n = 4) females as shown below (Table 6).

Gender	f	%
Male	6	60
Female	4	40

Table 6: Shown is the sex distribution of subjects included in the study.

Most of the subject in this study are ages 3 years old to 4 years old 40% (n = 4) with mean age distribution of 2.45 years old as shown in Table 7.

Age	f
1mo – 11 mos	1
1yo -2yo	2
2yo 1mo -3yo	3
3yo 1mo – 4yo	4

Table 7: Shown is the age distribution of subjects included in the study.

Majority of the patients are living in Quezon City 90% (n = 9) and 1 subject is outside Metro Manila as shown below (Table 8).

Residence	f	%
Quezon City, Metro Manila	9	90
Tanay Rizal	1	10

Table 8: Shown are the residence of subjects included in the study.

In terms of comorbidities, 60% (n = 6) have comorbidities in which 2 have bronchial asthma-controlled, pulmonary tuberculosis, umbilical hernia and hydrocele (Table 5). One patient is a 9-month-old with multiple comorbidities: global developmental delay, congenital hypothyroidism and nephrolithiasis as shown below (Table 9).

	Comorbidities	f	%
Yes		4	40
No		6	60

Table 9: Shown are the number of subjects with comorbidities.

The most prevalent symptoms presented by the subjects were cough 100% (n = 10), followed by fever 70% (n = 7), and colds 20% (n = 2) as shown below (Table 10).

Symptoms	f	%
Cough	10	100
Fever	7	70
Colds	2	20

Table 10: Shown is the common symptoms of subjects included in the study.

The severity of COVID 19 was based on the PIDS-P guidelines, [14] wherein 60% (n = 6) were moderate risk due to pneumonia in Chest X-ray (n = 5) and/or concomitant comorbidities (n = 1) as shown below (Table 11 & 12).

	Severity	f	%
Mild		4	40
Moderate		6	60

Table 11: Shown is the disease severity of subjects included in the study.

Chest X-ray	f	%
Pneumonia	5	50
Normal	3	30

Table 12: Shown is chest X-ray results of subjects included in the study.

Patients who were tagged COVID-19 moderate were hospitalized for treatment and close monitoring. None of the patients presented with severe disease and no accounted mortality during the course of the disease as shown below (Table 13).

Hospitalization	f	%
Yes	6	60
No	4	40

Table 13: Shown is number of hospitalized subjects included in the study.

Discussion

In this study, we successfully sequenced and characterized ten (10) SARS-CoV-2 samples from COVID-19 pediatric patients, collected between January 2021 and July 2023, using iSeq (Illumina, USA) in AFRIMS in Quezon City. All samples collected were Omicron variant.

This is congruent to the report of the Philippine Surveillance and Analysis of COVID-19 in Children Nationwide (SALVACION) database wherein COVID-19 cases in the Filipino pediatric population were Omicron variant and presented with mild-to-moderate symptoms (89.5%). [15] In correlation, all patients in this study presented with mild to moderate symptoms and generally had good prognosis and clinical outcomes.

SARS-CoV-2 and Pediatric Age Group

Generally, children have milder disease severity than adults. [3] Age becomes significant in terms of vaccine eligibility. [3,6] Several study supports that those with no vaccines are more susceptible to the disease compared to those with vaccines. [3,6,16] In a study by Yigit et al, unvaccinated and incompletely vaccinated children had a higher risk of COVID-19 infection compared to those who were fully vaccinated with an odd ratio of 4.8 and 1.83 respectively. [16] Since SARS-CoV-2 vaccines were only given to 5 years old and older, those 4 years old and below are susceptible to contact infection. [3,6] This was as also reflected in this study wherein majority are below 4 years old, 61% (n=147) and were not eligible to receive

the vaccine.

SARS-CoV-2, Residence, Sex and Comorbidities

The current circulating variant becomes the widely detected variant in a given period of time and place. In terms of the study setting, the Omicron variant invaded Philippines in the late December 2021, and it was also the most dominant variant in children. [15] In correlation, all 10 subjects with NGS in this study were infected by Omicron variant. Also, these samples were collected from July 2022 and onwards, hence detecting this variant was generally expected. [15]

In terms of sex, majority of the subjects in this study were male, 61% (n=147). The difference in sex distribution of the infectious disease is hypothesized to be due to the effect of hormones. [16] In a study by Zaher et al, the estrogen in female enhances T cell activity, stimulates interferon production, increase growth of B cells and antibody production. [16] All of these boost female's immune system and became a protective mechanism against infection. In contrast, the presence of androgen makes males more susceptible to viral infection. [16] An interesting finding that was recently discovered proposed that expression of TMPRSS2 receptors in peumocytes is androgen-dependent. [17] TMPRSS2 expression is an important enzyme in SARS-CoV-2 cell entry. [17] Thus, abundant testosterone level in male directly elevates TMPRSS2 expression therefore increasing the probability of viral entry. [17]

Lastly, the presence of comorbidities greatly affects the COVID-19 severity. [18,19] This was supported by a meta-analysis by Tsankov et al and Widjanarko et al, wherein those patients with comorbidities have higher risk of developing severe disease with an odd ratio of 1.79 and 4.07 respectively. [18,19] Moreover, studies showed that COVID-19 patient with comorbidities has a higher risk of mortality with relative risk ratio of 2.81. [18] In correlation, in this study 24% (n=12) of patient classified as moderate COVID-19 have preexisting comorbidities, supporting finding that comorbidities affects patient outcomes and prognosis.

SARS-CoV-2 Variants and Disease Severity

Reports on SARS-CoV-2 revealed that the Gamma and Delta waves lead to severe diseases in children. [20] This was seen in the massive increase in number of admitted children due to COVID- 19 during these 2 waves. On the other hand, Omicron was observed to be more contagious but has lesser disease severity. [22,21] This was also observed in this study wherein all subjects (n=10) were infected by Omicron variant and generally have lesser disease severity and good clinical outcomes.

Omicron, the most dominant in children

The dominance of Omicron variant in pediatric population was theorized to be due to its mode of cellular entry. According to the PIDS-P COVID-19 guidelines, in contrast to adults, pediatric population are more resistant to former SARS-CoV-2 variants due to children's innate low amount of TMPRSS2 in type I alveolar epithelial cells, and ACE2 receptors in type II alveolar epithelial cells [14]; and both are vital for viral entry. [14] However, the Omicron variant is unique, that it has higher affinity to ACE2 receptor and solely needs this receptor to invade a host, thus making unvaccinated pediatric patient more vulnerable. [21]

Aside from efficient viral entry, the Omicron adapt a more advantageous behavior to spread. A study of Montarrayos et al revealed that Omicron has 6 times higher incidence rate than other variants due to higher effective reproduction number (Rt). [22] Interestingly, a study by Chatterjee et. al also shows that Omicron viral load is unique, in that they are concentrated in the upper airway, mostly in the nose, windpipe, and throat, but less in the lower respiratory system. [23] This location makes it easier for the virus to be incorporated to respiratory droplets and spread more efficiently. Coupling a faster viral reproduction and concentrated location in the upper respiratory tract, makes omicron more contagious than the previous variants. [23]

Lastly, the most important factor in Omicron's virulence is the viral structure itself. It was proposed by the study of that Omicron's higher mutation rate compared to other variants makes them variant of concern. [24] This was supported by a study of wherein Omicron variant has the highest number of alteration sites, with a staggering 60 substitutions, insertions, and deletions, whereas the second most diverse variant, Delta, has only 22 mutations. [25] This diversity in its protein enables them to take an alternative cell entry pathway (endosomal fusion pathway) and subsequently escaping the host immune system more efficiently. The combined aforementioned characteristics of Omicron could explain why all the subjects in this study are infected by the said variant, as it has more mutations, advantageous behavior to spread and with the most viral load. [25]

Omicron is Less Virulent

But despite its higher infection rate, Omicron infected patients had mild to moderate symptoms. As supported by several published studies done in Miami, Ohio and Mexico, [20,21,22] in comparison to Gamma and Delta variants, Omicron variant caused less severe disease in pediatric population despite its higher incidence rate. Like in this study, all patients presented with cough, colds, fever and loose stool but majority did not require hospital admission. However, there are other contrasting studies on Omicron's lesser disease severity. A study by Lam et. al., revealed that Omicron caused croup-like symptoms in pediatric patient in Hongkong comparing to pre-omicron era leading to more admission cases. [26] It was proposed that the possible explanations for greater morbidity is due to its higher transmissibility in younger populations compared with other variants [21], along with children's lack of eligibility for SARS- CoV-2 vaccines, this makes this population more susceptible to omicron. [20] Likewise, a study by Han et. al., also observed that omicron cases had higher peak fever and higher rate of croup and febrile seizures compared with the previous waves. [17]

Despite this contrasting studies, strong evidences from a systematic review composed of researches from 52 countries worldwide concluded that comparing to Delta variant, Omicron still pales in disease severity. [27] There are no established evidence explaining its lesser disease morbidity, but a study of Mukherjee et. al hypothesized that this is due to omicron's low effectivity in counteracting the host cell interferon response. [28] Interferon, a cytokine that plays an important role in the activation of body's immune defenses. [28] Thus, Omicron's failure in suppressing interferon, enables the innate immune system to easily recognize and destroy the virus. [28]

Omicron and the Increasing Seizure Incidence

In comparison to other variants, several studies show that Omicron wave leads to more seizure incident. [29,30] This was also congruent in this study wherein 10% of the patient had seizures. Studies of Kim et. al. and Tokuyama et. al., hypothesized that interferon is mainly involved in the increasing incidence of febrile seizure in Omicron wave. [29,30] Because omicron had higher viral replication, this induces higher concentrations of host interferons, a principal component of an innate immune response. 30 This higher amount of interferon then develops to a more intense innate immune reactions, thus leading to higher grade fever and subsequently higher risk of febrile convulsions. [29,30]

SARS-CoV-2 in Children, the Role of Energy Allocation

There are different researches proposing hypothesis on pediatric resistance to severe COVID-19. Although there are no concrete and established evidences yet, there are theories that became popular in the field of immunology. Since pediatric population manifests different physiology compared to adults, they respond differently in encountering diseases. [31] Human are born defenseless with immature systems, hence, there is a strong evolutionary pressure to mature over time. [31] And one important aspect that is unique in pediatric population, is the rapid growth early in life. [31] This then leads to dilemma of energy allocation trade off, is it whether to invest this energy to produce immune defenses or to prioritize growth? [32] Children's body will likely favor growth unless the pathogen will lead to life-threatening outcomes. [32] In the study of Brodin, he called this phenomenon, disease tolerance in children, an immune defense strategy activated when the immune response to a pathogen is more damaging than the pathogen itself. [33] The body prevents production and activation of immune response, and tolerates the pathogen rather than resisting it, thereby allocating most of the energy to growth and development. [33] Brodin then concluded that there are higher energy allocation to growth in younger population compared to adult. [33] This is one of the hypothesis explaining why children have lesser COVID-19 disease severity compared to adults, children produce less cytokines and inflammatory agents. [33] Interestingly, male also has higher energy allocation to growth compared to female. [34] This is congruent to our study, wherein 61% (n=147) were less than 4 years old and 61% (n=147) were male, and majority presented with mild symptoms.

Limitation of Next Generation Sequencing

Overall, this study was relatively limited by a small number of populations, with just merely ten (10) sequences, this make correlation and drawing conclusions difficult. This limitation is due to the rarity of NGS and its cost effectiveness, it is not routinely done, moreso in the developing countries like the Philippines. Despite the small sample, this study supported the findings of other researches that

preceded it, a valuable information in understanding the spread and evolution of SARS-CoV-2 in pediatric population in the Philippines. This novel coronavirus was widely studied during the pandemic, but despite the rampant chaos it brought in the community, there were few published literatures of variant outcomes in pediatric population, as well as the aftermath of its rapid evolution.

Conclusion

From January 2021 to July 2023, a total of 630 pediatric patients who got tested for SARS- CoV-2 RT-PCR were confirmed to have SARS-CoV-2. Two hundred thirty-nine (239) were included in this study. Among the study population, 61.51% were male, more than 60% were less than 4 years old and residing in Quezon City. In terms of clinical presentation, 78% had mild symptoms, 20% had radiologic findings of pneumonia, 40% presented with fever, 12% with diarrhea, and 8% with dyspnea. Ten percent (10%) of patient had concomitant seizure disorder. More than 70% were admitted to the hospital and all of the study subjects recovered from the illness.

Only ten (10) samples were subjected to next generation sequencing and all were Omicron variant. Despite the homogenous variant, the isolated viruses have different lineages with some tagged as variant of interest and variant under monitoring. Omicron's dominance may be attributed to its abundant mutation, high effective reproduction number and higher affinity to ACE2 receptors compared to other variants. Also, majority of the patients presented with mild symptoms such as fever and cough, and generally had good clinical outcomes. This could be associated to Omicron's inefficient suppression of the host cell interferon response as opposed to other variants, making it more susceptible to the innate immunity defenses.

Recommendation

Based on the study conclusion, the following recommendation were deduced:

1. To continue using next generation sequencing as part of work-up especially in complicated, unknown and severe cases of SARS-CoV-2 and other viral infections.
2. To correlate viral sequence of the SARS-CoV-2 to previous endemic coronavirus in the Philippines and identify mutated sequences that may correlate to its novelty and virulence.

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