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Original Article

The epidemiology and phylogenetic trends of Omicron subvariants from BA.5 to XBB.1 in Taiwan

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ABSTRACT

Background: Omicron, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant, entered Taiwan at the end of 2021. The Taiwanese government ended its "zero-COVID" policy in March 2022. Multiple coronavirus disease 2019 (COVID-19) outbreaks began in April 2022. We monitored the replacement of Omicron subvariants after BA.1/BA.2 and analyzed their correlation with COVID-19 outbreaks.
Methods: We collected SARS-CoV-2 real-time qRT–PCR-positive nasopharyngeal swabs from Kaohsiung Medical University Hospital (KMUH), Kaohsiung City, Taiwan, and performed sequencing for specimens exhibiting a cytopathic effect in Vero E6 cells to determine their clades and lineages. We analyzed the medical records of COVID-19 patients and identified hospitalization risk factor(s). We retrieved SARS-CoV-2 sequences identified in Taiwan from GISAID and analyzed their correlation with COVID-19 data from the Taiwan Centers for Disease Control.
Results: We analyzed the phylogenesis of KMUH-47 to KMUH-104 (SARS-CoV-2 isolates identified herein) and all of the Omicron subvariants from BA.5 to XBB.1 (n = 1930). Age and comorbidities were hospitalization risk factors. Men generally exhibited a greater fatality rate than women. COVID-19-related deaths predominantly occurred in individuals over 70 years old. The COVID-19-related case fatality rate increased as nucleotide (NT) and amino acid (AA) substitutions increased. The number of COVID-19-related cases and deaths progressively decreased with each outbreak between August 2022 and October 2023.
Conclusion: Hospitalization was associated with age and the presence of comorbidities. COVID-19-related fatality was linked to sex, age, and the accumulation of NT and AA substitutions in emerging Omicron subvariants.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), originated in Wuhan, Hubei Province, China, in December 2019 [1]. Although the modes of transmission and infection of the virus were

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identified in 2020 [2–5], the debate over whether the virus originated naturally or resulted from a laboratory leak continues in 2024 [6]. The World Health Organization (WHO) declared COVID-19 a global pandemic on March 11, 2020 [7]. Since its initial identification, COVID-19 has evolved into a global pandemic, profoundly impacting public health, healthcare systems, and societies worldwide. The COVID-19 pandemic has triggered extensive research, and the evolution of the virus has led to the emergence of various SARS-CoV-2 variants, which have been classified as variants of interest (VOIs), variants of concern (VOCs), and variants under monitoring (VUMs). In particular, the emergence of VOCs, including Alpha, Beta Delta and Omicron, has raised concerns about potential high transmissibility, increased severity, and immune escape [8].

Since the emergence of the Omicron variant in late 2021, it has proven to be more contagious than previous VOCs, while also reducing hospitalization rates and presenting milder symptoms. These characteristics have led many to view Omicron as a potential "natural vaccine" [9]. New Omicron subvariants continue to accumulate additional mutations in the Spike protein and other genes [10], which enhance Omicron's ability to evade neutralizing antibodies acquired from prior infections and/or vaccinations, contributing to ongoing global concern and pandemic spread [11–13]. The various Omicron subvariants include BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), BA.3 (B.1.1.529.3), BA.4, BA.5, and their descendant lineages. Recently, subvariants such as BQ.1, BQ.1.1, BA.4.6, BF.7, BA.2.75.2, XBB.1, and BF.7 have also drawn global attention [14].

A previous study indicated that BA.1 and BA.2 and their subvariants entered Taiwan in December 2021 and January 2022, respectively. However, until March 2022, neither of the Omicron sublineages caused a COVID-19 outbreak. The study also indicated that BA.2.3.7 emerged in Taiwan in March 2022, became the dominant Omicron subvariant between April 2022 and August 2022, and may have contributed to the largest COVID-19 outbreak since 2020. The severity of Omicron-related COVID-19 has increased over time, driven by the accumulation of genetic variations resulting in amino acid changes [15,16]. Omicron subvariants continue to cause outbreaks globally and within Taiwan. However, comprehensive research reports on the epidemiology of COVID-19 in Taiwan since August 2022 are lacking.

Genome sequencing of positive samples can help identify various virus variants, enabling the mapping of cases in a specific area [14]. In this study, we aimed to investigate and integrate epidemiological information on the COVID-19 epidemic in Taiwan by monitoring the transmission dynamics of the Omicron sublineages and their subvariants and analyzing COVID-19 data from the Taiwan Centers for Disease Control (CDC), providing a deeper understanding of the ongoing COVID-19 pandemic. We hope that the results of this effort provide valuable insights and contribute to the global fight against the COVID-19 pandemic.

Materials and methods

Sample collection and ethics statement

We collected nasopharyngeal swabs from volunteers with suspected COVID-19 in Viral Transport Medium (VTM) (Creative Life Science, Taiwan) from August 2022 to October 2023. This study was reviewed and approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH) (approval no. KMUHIRB-E-I-20200013).

Detection of the SARS-CoV-2 genome by real-time qRT–PCR

Total RNA from the swab-VTM was extracted as described in our previous study [15,17]. The presence of the SARS-CoV-2 genome was confirmed using the Cobas SARS-CoV-2 & Influenza A/B test, which targets both the ORF1 a/b nonstructural region and the nucleocapsid protein-encoding gene unique to SARS-CoV-2, on a Liat Analyzer (Roche Molecular Systems, Inc., NJ, USA). The results were automatically interpreted.

SARS-CoV-2 culture

SARS-CoV-2 culture was performed following the procedures outlined in our previous studies [15,17–19]. SARS-CoV-2 propagation was subsequently confirmed through qRT–PCR.

RNA library construction, whole-genome sequencing (WGS), and lineage identification of whole-genome sequences

Viral RNA extraction, RNA library construction, WGS, and sequence analysis were performed on the swab-VTM samples following the methodologies described in our prior studies [15,17–19]. The clade and lineage of the full-length sequences (approximately 2.9 kb) were determined using Nextclade v3.0.0 (https://clades.nextstrain.org/) [20]. The output lineages were subsequently verified using the Lineage Mutation Tracker from outbreak.info (https://outbreak.info/) [21].

PCR amplification, Sanger sequencing and lineage identification

The Spike gene fragments were amplified for Sanger sequencing using the methodologies outlined in our previous studies [15,17–19]. The primer pairs used are detailed in Supplementary Table 1. Sequence variation was analyzed using Molecular Evolutionary Genetics Analysis (MEGA 11) software (Philadelphia, USA) [22], by comparing the sequences with those of the wild-type virus (Wuhan-Hu-1/2019, MN908947). The Pango lineage [23] of SARS-CoV-2 was identified on the basis of Spike gene mutation information derived from outbreak.info [21]. Since the mutations in the Spike gene are somewhat similar in Omicron (B.1.1.529) strains among sublineages [24], specific mutations were included to distinguish BA.4 (B.1.1.529.4)/BA.5 (B.1.1.529.5)/BF.7 (B.1.1.529.5.2.1.7). The D3N mutation of the M gene was used to differentiate between BA.4 and BA.5, whereas deletion and missense mutations of the N gene at amino acid positions 30–33 were used to differentiate between BA.5 and BF.7 (Supplementary Table 2).

SARS-CoV-2 sequence submission, download, and analysis

The SARS-CoV-2 sequences generated in this study were uploaded to GISAID EpiCoV (https://www.gisaid.org/) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The sequences identified in Taiwan between 1 August 2022 and 31 October 2023 were accessed from GISAID. The analysis in this study included a total of 1930 SARS-CoV-2 sequences. Of these, only 192 records specified the city where the sample was collected, with 13 samples from Taipei City and 179 from Tainan City. The SARS-CoV-2 isolates in this study (N = 55) were all obtained from COVID-19 patients in Kaohsiung City, except for KMUH-59S, which was from a patient in Pingtung County. The remaining 1683 samples did not include information about the city of collection. SARS-CoV-2 sequence analysis and sequence placement on the existing phylogenetic tree were implemented by using Nextclade v3.0.0 [20].

Retrieval of Taiwan and global COVID-19 data

COVID-19 data in Taiwan were obtained from a web-based notifiable disease surveillance system (https://nidss.cdc.gov.tw/en/Home/Index) and daily press conferences conducted by the Central Epidemic Command Center (CECC), Taiwan [25]. Global COVID-19

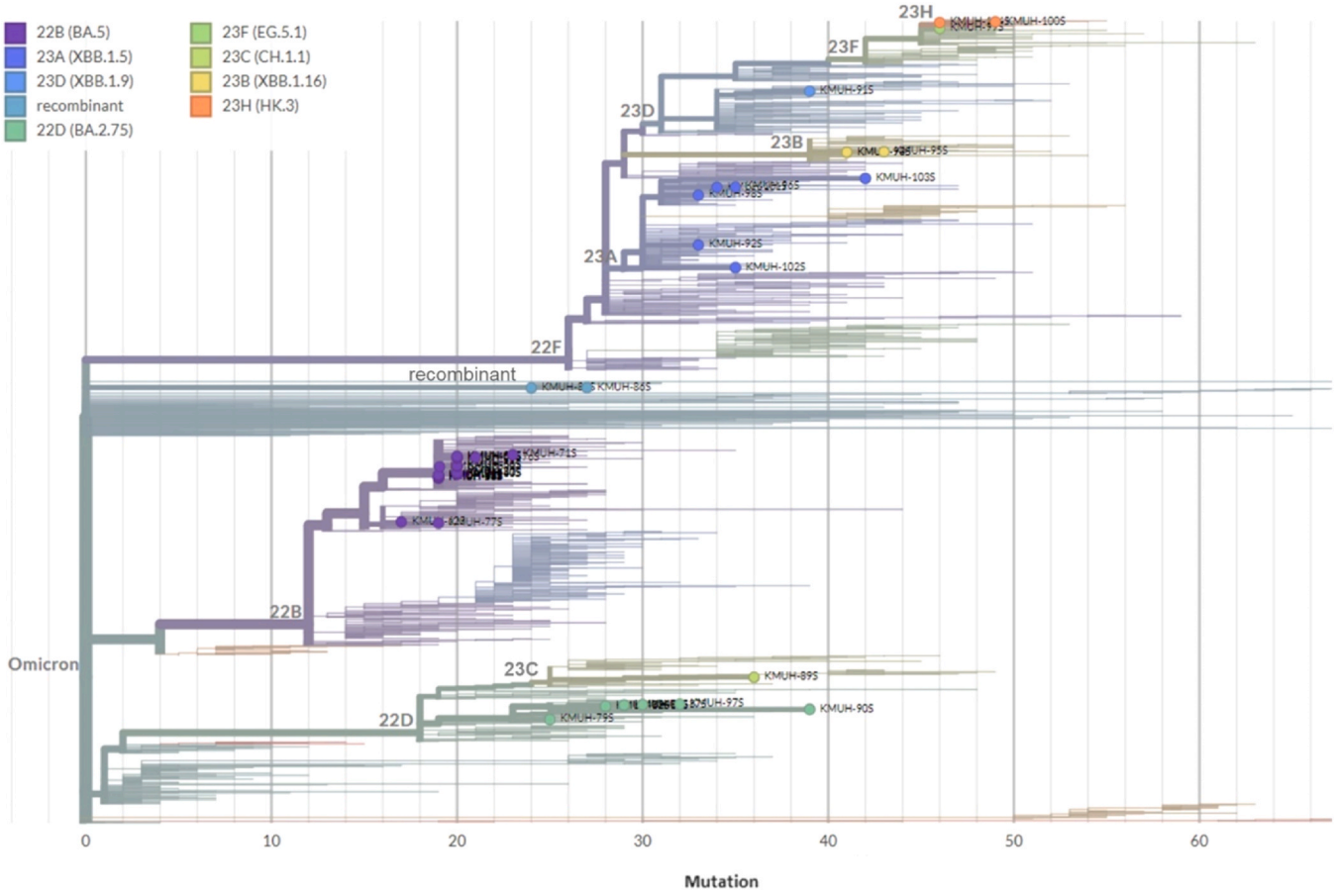

Fig. 1. Phylogenetic analyses of the SARS-CoV-2 Omicron subvariants identified and sequenced in this study using Nextclade. The Spike gene of the SARS-CoV-2 Omicron variants identified in this study, designated with names starting with KMUH in the figure, was analyzed using Nextclade, and a partial phylogenetic tree derived from placement results on a global SARS-CoV-2 phylogenetic tree is presented.

data were retrieved from Our World in Data (https://ourworldindata.org/). Notably, these data are publicly accessible.

Statistical analysis

We performed statistical analysis of COVID-19 data using MedCalc for Windows version 22.018 (MedCalc Software, Ostend, Belgium). The results were considered statistically significant at $P < 0.05$.

Results

Monitoring SARS-CoV-2 in Taiwan from August 2022 to October 2023

Among the collected nasopharyngeal swabs, SARS-CoV-2 genomic RNA was detected in 194 swab-VTM samples using real-time qRT–PCR. The integrity of the SARS-CoV-2 genome in these swab-VTM samples was evaluated through virus culture. CPEs were observed in 55 out of 194 swab-VTM samples. Fifty-five swab-VTM samples that induced a CPE were subsequently subjected to sequencing for either the full-length genome or the Spike gene. The results of phylogenetic placement are shown in Fig. 1, and the demographic information, including the clade and Pango lineage, of these sequences is shown in Table 1. The analysis revealed that all of the identified isolates were Omicron subvariants; 54.5 % (n = 30) were classified as clade 22B, 16.4 % (n = 9) as clade 22D, 10.9 % (n = 6) as clade 23 A, 5.5 % (n = 3) as clade 23B, 3.6 % (n = 2) as clade 23 H, 3.6 % (n = 2) as recombinant, 1.8 % (n = 1) as clade 23 C, 1.8 % (n = 1) as clade 23D, and 1.8 % (n = 1) as clade 23 F. The epidemic outbreak trend of these subvariants was basically consistent with the global trend, but due to geographical factors, the timing occurred slightly later. Among the 32 hospitalized COVID-19 patients, 12 were female and 20 were male, with an average age of 53.8 (± 31.0) years and a median age of 64 years (Table 1). Among the 23 nonhospitalized COVID-19 patients, 15 were female and 8 were male, with an average age of 25.9 (± 26.4) years and a median age of 22 years. Hospitalized patients were significantly older than nonhospitalized patients were ($P < 0.001$), but their qRT–PCR cycle threshold values were not significantly different. Thirty out of 32 hospitalized patients had one or more comorbidities, whereas only 8 out of 23 nonhospitalized patients had comorbidities. These findings indicate that comorbidities serve as risk factors for hospitalization among COVID-19 patients ($P < 0.0001$) (Table 1). Two patients died after contracting COVID-19. Both were infected with a BA.5 strain. One patient was a 93-year-old woman with comorbidities such as hypertension, chronic kidney disease, and Parkinson's disease. The other patient was a 69-year-old man with comorbidities such as type 2 diabetes, peripheral arterial occlusive disease, and chronic hepatitis B.

Omicron-related COVID-19 between April 2022 and October 2023 in Taiwan

To provide a comprehensive overview of the epidemic situation, we examined COVID-19-related data from Taiwan spanning from April 2022 to October 2023. The data obtained from the Taiwan CDC revealed fluctuations in the number of monthly COVID-19 cases, ranging from 120 thousand to 1.98 million, during the period between April 2022 and March 2023. Notably, approximately 96.8 % to

Table 1
Demographic information on the SARS-CoV-2 subvariants identified and sequenced in this study.

seqName[a]	Clade	Pangolin lineage	Alias	Sex	Age	Ct[b]	Comorbidity	Hospitalization	Collection Date[c]	GISAID[d]	GenBank
KMUH−47	22B	BA.5	BA.5	M	6	11	-	+	2022/8/8	17675379	OQ931782
KMUH−48S	22B	BA.5	BA.5	M	44	12	-	-	2022/8/15	17660878	OQ931783
KMUH−50S	22B	BA.5	BA.5	F	1	10	+	+	2022/8/24	17660879	OQ931784
KMUH−51S	22B	BA.5	BA.5	M	1	12	+	+	2022/8/31	17660886	OQ931785
KMUH−53S	22B	BA.5	BA.5	F	45	11	-	-	2022/9/3	17660893	OQ931786
KMUH−54S	22B	BA.5	BA.5	F	37	11	-	-	2022/9/5	17661416	OQ931787
KMUH−55S	22B	BA.5	BA.5	F	34	11	+	-	2022/9/7	17661417	OQ931788
KMUH−56S	22B	BA.5	BA.5	M	5	11	+	-	2022/9/10	17661418	OQ931789
KMUH−57S	22B	BA.5	BA.5	M	75	12	+	+	2022/9/11	17661420	OQ931790
KMUH−58S	22B	BA.5	BA.5	F	63	13	+	+	2022/9/12	17661426	OQ931791
KMUH−59S	22B	BA.5	BA.5	M	81	12	+	+	2022/9/13	17661506	OQ931792
KMUH−60S	22B	BA.5	BA.5	F	64	12	+	+	2022/9/13	17661517	OQ931793
KMUH−61S	22B	BA.5	BA.5	F	65	10	-	-	2022/9/19	17661518	OQ931794
KMUH−62S	22B	BA.5	BA.5	F	59	11	+	+	2022/9/21	17661520	OQ931795
KMUH−63S	22B	BA.5	BA.5	M	0	11	+	-	2022/9/22	17661521	OQ931796
KMUH−64S	22B	BA.5	BA.5	M	53	10	+	+	2022/9/23	17661522	OQ931797
KMUH−65S	22B	BA.5	BA.5	M	0	11	-	-	2022/9/26	17661523	OQ931798
KMUH−66S	22B	BA.5	BA.5	F	2	10	+	+	2022/9/28	17664268	OQ931799
KMUH−67S	22B	BF.7	BA.5.2.1.7	F	53	8	-	-	2022/10/2	17664269	OQ931800
KMUH−68S	22B	BA.5	BA.5	F	82	11	+	+	2022/10/3	17664270	OQ931801
KMUH−69S	22B	BA.5	BA.5	M	77	11	+	+	2022/10/3	17664271	OQ931802
KMUH−70	22B	BA.5.2.1	BA.5	F	75	10	-	-	2022/10/5	17675387	OQ931803
KMUH−71S	22B	BA.5.2.49	BA.5	F	28	11	+	-	2022/10/10	17664295	OQ931804
KMUH−72S	22B	BA.5	BA.5	F	0	11	-	-	2022/10/11	17664296	OQ931805
KMUH−73S	22D	BN.1	BA.2.75.5.1	M	15	11	+	-	2022/10/12	17664297	OQ931806
KMUH−75S	22B	BA.5	BA.5	F	84	13	+	-	2022/11/15	18108904	OR457659
KMUH−76S	22B	BA.5	BA.5	F	93	12	+	+	2022/11/16	17664298	OQ931807
KMUH−77S	22B	CC.1	BA.5.3.1.1.1.2.1	M	56	14	+	+	2022/12/3	17664299	OQ931808
KMUH−78S	22B	BA.5	BA.5	M	74	9	+	+	2022/12/17	17664470	OQ931809
KMUH−79S	22D	BN.1.1	BA.2.75.5.1.1	F	66	13	+	+	2022/12/22	17664471	OQ931810
KMUH−80S	22B	BA.5	BA.5	M	69	11	+	+	2023/1/1	17664472	OQ931811
KMUH−81S	22B	BA.5	BA.5	M	56	12	+	+	2023/1/9	17664473	OQ931812
KMUH−82S	22D	BN.1	BA.2.75.5.1	M	68	12	+	+	2023/1/23	17664474	OQ931813
KMUH−83S	22D	BN.1	BA.2.75.5.1	F	1	10	+	+	2023/2/11	17664475	OQ931814
KMUH−84S	recombinant	XBF / CJ.1	XBF/BA.2.75.3.1.1.1.1	M	68	11	-	+	2023/2/12	17664512	OQ931815
KMUH−85S	22D	BN.1	BA.2.75.5.1	M	82	11	+	+	2023/3/8	17664513	OQ931816
KMUH−86S	recombinant	XBF / CJ.1	XBF/BA.2.75.3.1.1.1.1	M	87	13	+	+	2023/3/17	17664535	OQ931817
KMUH−87S	22D	BN.1	BA.2.75.5.1	M	1	15	+	+	2023/4/10	18108906	OR457660
KMUH−88S	22D	BN.1	BA.2.75.5.1	F	1	15	-	-	2023/5/2	18108908	OR457661
KMUH−89S	23 C	CH.1.1.1	BA.2.75.3.4.1.1.1.1.1	F	24	12	-	-	2023/5/8	18108909	OR457662
KMUH−90S	22D	BN.1	BA.2.75.5.1	M	35	17	+	+	2023/5/12	18108911	OR457663
KMUH−91S	23D	XBB.1.5.17	XBB.1.5.17	F	64	12	+	+	2023/5/23	18108912	OR457664
KMUH−92S	23 A	XBB.1.5.12	XBB.1.5.12	F	1	12	-	-	2023/6/7	18108914	OR457665
KMUH−93S	23B	XBB.1.16	XBB.1.16	M	40	13	+	+	2023/6/22	18134631	OR482172
KMUH−94S	23B	XBB.1.16	XBB.1.16	F	2	15	+	-	2023/6/26	18134633	OR482173
KMUH−95S	23B	XBB.1.16	XBB.1.16	M	3	13	-	-	2023/7/3	18134634	OR482174
KMUH−96S	23 A	EG.1	XBB.1.9.2.1	M	1	12	+	+	2023/7/5	18134636	OR482175
KMUH−97S	22D	BN.1	BA.2.75.5.1	F	5	13	-	-	2023/7/9	18134637	OR482176
KMUH−98S	23 A	XBB.1.5	XBB.1.5	F	75	14	+	+	2023/7/17	18134638	OR482177
KMUH−99S	23 F	EG.5.1	XBB.1.9.2.5.1	M	4	8	-	-	2023/8/14	18321292	OR605598
KMUH−100S	23 H	HK.3.3	XBB.1.9.2.5.1.1.3.3	M	91	12	+	+	2023/8/26	19022834	PP565348
KMUH−101S	23 A	EG.1	XBB.1.9.2.1	F	49	11	+	-	2023/9/5	19022835	PP565349
KMUH−102S	23 A	FD.2	XBB.1.5.15.2	F	47	20	+	+	2023/9/6	19022836	PP565350
KMUH−103S	23 A	XBB.1.5.10	XBB.1.5.10	M	85	11	+	+	2023/9/14	19022850	PP565351
KMUH−104S	23 H	HK.3	XBB.1.9.2.5.1.1.3	M	22	15	-	-	2023/10/23	19022851	PP565352

[a] The sequence names associated with the SARS-CoV-2 Omicron variants uncovered in this study start with KMUH. A name with an "S" indicates that is a *Spike* gene sequence. Otherwise, it is a full-length sequence.
[b] Cycle threshold (Ct) values for SARS-CoV-2 genome real-time qRT-PCR.
[c] Swab collection date, YYYY/MM/DD.
[d] EPI_ISL_number
Ct; cycle threshold.

99.9 % of these cases were classified as autochthonous (Table 2). The number of monthly COVID-19 cases decreased to approximately three thousand between April and October 2023. This apparent decline was attributed to the Taiwan CDC's alteration in the definition of notifiable COVID-19 cases. Visual analysis of the distribution of COVID-19 cases by age revealed a bell curve, indicating that cases were predominantly concentrated in the 20–49 year age group for both men and women (figure not shown). Both COVID-19-related deaths and the CFR continued to increase with age, for both men and women. In total, individuals aged over 70 years accounted for 7.4 % of all COVID-19 cases but accounted for 79.0 % of the total deaths. Overall, men had a higher CFR than women did (1.8 to 3.3-fold). However, a significant difference was found only after the age of 20 years ($P < 0.05$) (Table 3).

Furthermore, three COVID-19 epidemic waves were noted between April 2022 and March 2023, each lasting approximately four months. Notably, the cumulative number of COVID-19 cases in each wave continuously decreased (Fig. 2A). Furthermore, between April 2022 and March 2023, 40- to 44-year-old patients presented the greatest number of COVID-19 cases (Fig. 2B). Notably, the confirmed cases between April and October 2023 predominantly involved individuals over 70 years old (Fig. 2C).

Table 2
Monthly COVID-19 data between April 2022 and October 2023.[a].

Year	Month	Autochthonous		Imported		Total		Death[c]		CFR[d] %	
2022	April	116,605		3795		120,400		119		0.10 %	
	May	1982,913		1632		1984,545		3553		0.18 %	
	June	1675,743		1735		1677,478		3696		0.22 %	
	July	795,620		5545		801,165		1372		0.17 %	
	August	729,723		7781		737,504		831		0.11 %	
	September	1155,436		6847		1162,283		1483		0.13 %	
	October	1240,827		1513		1242,340		1960		0.16 %	
	November	580,354		1354		581,708		989		0.17 %	
	December	541,358		2542		543,900		973		0.18 %	
2023	January	688,394		8443		696,837		1515		0.22 %	
	February	479,072		6597		485,669		1405		0.29 %	
	March[b]	181,041	1344	185,417	4	184,069	1348	504	68	0.27 %	5.04 %
	April	2889		15		2904		311		10.71 %	
	May	5680		10		5690		566		9.95 %	
	June	7108		8		7116		1038		14.59 %	
	July	4164		4		4168		862		20.68 %	
	August	1683		4		1687		318		18.85 %	
	September	1132		9		1141		165		14.46 %	
	October	1184		2		1186		143		12.06 %	

[a] COVID-19 data were retrieved from the web-based notifiable disease surveillance system maintained by the Taiwan CDC. Source of data: https://nidss.cdc.gov.tw/nndss/disease?id= 19CoV.
[b] People who tested positive for SARS-CoV-2 before March 19, 2023, were notifiable (left side of each column). Effective March 20, 2023, mild COVID-19 cases were exempt from reporting and isolation, and only patients who met the criteria for moderate or severe COVID-19 were notifiable (right side of each column).
[c] Numbers of COVID-19-related deaths after March 20, 2023, were retrieved from the Taiwan CDC news archive. Source of data: http://at.cdc.tw.
[d] CFR: Case fatality rate.

Table 3
Distribution of the age and sex of confirmed COVID-19 patients and COVID-19-related deaths between April 2022 and October 2023.[a].

Age	Sex	COVID-19 cases	Deaths	CFR (%)	*P* value[b]
0 –9	Female	485,826	14	0.003	0.0842
	Male	534,501	27	0.005	
10 –19	Female	518,407	4	0.001	0.1592
	Male	576,211	10	0.002	
20 –29	Female	860,391	17	0.002	0.0070
	Male	813,905	35	0.004	
30 –39	Female	977,591	40	0.004	< 0.0001
	Male	835,106	79	0.009	
40 –49	Female	973,384	152	0.016	< 0.0001
	Male	748,844	304	0.041	
50 –59	Female	688,058	332	0.048	< 0.0001
	Male	510,795	812	0.159	
60 –69	Female	543,682	873	0.161	< 0.0001
	Male	411,841	1901	0.462	
70 +	Female	422,183	7828	1.854	< 0.0001
	Male	340,438	9447	2.775	

[a] Source of data: http://at.cdc.tw.
[b] Two-way chi-square test.

Distribution of Omicron subvariants between August 2022 and October 2023 in Taiwan

To determine the distribution of clades and subvariants in Taiwan between August 2022 and October 2023, the sequences were accessed and downloaded from GISAID (n = 1930). The results indicated that 21 % of the SARS-CoV-2 isolates belonged to the former VOC Omicron, whereas the others represented newly emerging VOIs or VUMs (Fig. 3A). The distributions of 347 Omicron subvariants are provided in Supplementary Table 3. Previous findings suggested that BA.2.3.7 dominated from April to August 2022 [15]. The findings indicated a transition, with BA.5.x dominating from September 2022 to January 2023, followed by BA.2.75.x, XBB.1.5.x, XBB.1.9.x, XBB.1.16.x and many other variants (Fig. 3B). The results of phylogenetic placement, clade, and Pango lineage analyses for these sequences are presented in Fig. 3C and Supplementary Table 3. Nucleotide (NT) and amino acid (AA) substitutions in the SARS-CoV-2 genome were continuously enriched during that period. NT substitutions accumulated from an average of 63 substitutions per genome to 108.3, whereas AA substitutions accumulated from an average of 47.9 to 74.9 per genome. NT and AA deletions accumulated from 52.0 and 11.2 on average, respectively, from August to November 2022 to average values of 56.4 and 13.1, respectively, from December 2022 to October 2023 (data not shown). When these sequences were compared with the BA.2 sequence, the trends in NT and AA substitutions were similar to the above results (Fig. 3D). Our findings revealed a positive correlation between accumulated NT and AA substitutions in Omicron subvariants and an elevated gross CFR from August 2022 to February 2023. The R-squared value for AA substitution versus CFR was 0.759 (BA.2 as a reference, r^2 =0.878). Similarly, from April to July 2023, NT and AA substitutions in Omicron subvariants correlated with the CFR of moderate to severe COVID-19 (Fig. 3D and Table 2). The R-squared value for AA substitutions versus CFR was 0.835 (BA.2 as a reference, r^2 =0.809).

COVID-19 fluctuations and vaccination status

We previously noted a marked decrease in the cumulative CFR during the BA.2.3.7 variant outbreak from April to August 2023 as the vaccination program advanced [15]. However, we have not investigated the interplay among vaccination status, subvariant replacement, and fatality. Before BA.2.3.7, Taiwan's vaccination rates were 83.4 %, 78.2 %, and 49.5 % for the first, second, and third doses, respectively (Fig. 4A and B). Nevertheless, herd immunity from previous infections and vaccinations proved insufficient against BA.2.3.7 (Fig. 4B). Two epidemic waves correlated with BA.2.3.7 and BA.5.x emergence (Fig. 4B). The government introduced a BA.4/BA.5 vaccine, but two additional waves occurred with BA.2.75.x and XBB.1.x (Figs. 4A, 4B, and Table 2). In addition to the three death waves associated with these epidemic waves (Fig. 4B), another smaller death wave occurred with XBB.1.5, XBB.1.16, and XBB.1.9.2 from May to September 2023, after which the XBB.1.5 vaccine was introduced in late September 2023 (Figs. 4A and 4B).

Discussion

After SARS-CoV-2 entered Taiwan in January 2020, the government adopted a "zero-COVID" policy. Since January 2022, BA.1 and BA.2 have spread uncontrollably in Taiwan [15]. Research has shown

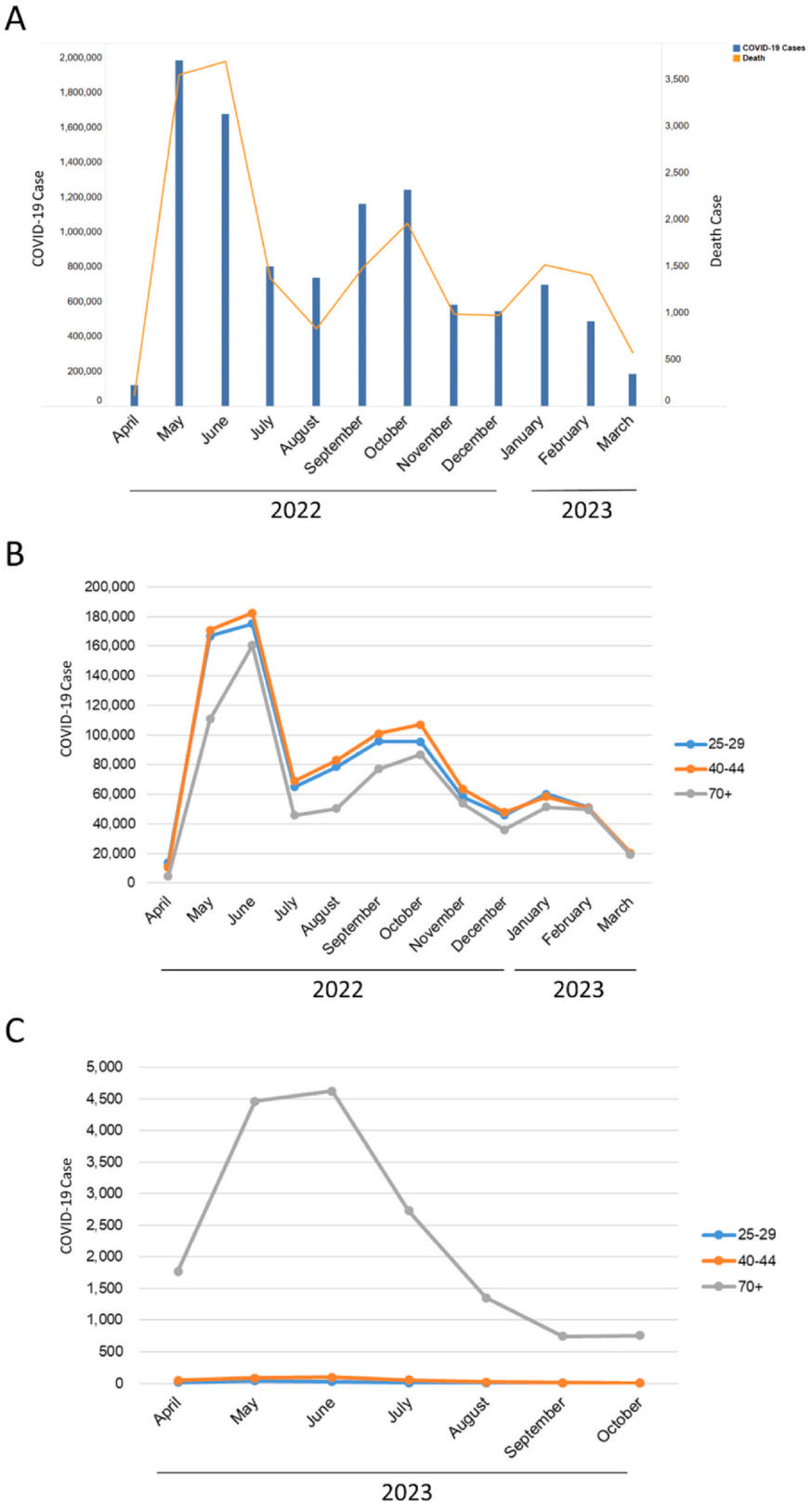

Fig. 2. Distribution of COVID-19 cases, fatal cases, and COVID-19 cases by age groups. (A) Distribution of COVID-19 cases and COVID-19-related deaths from April 2022 to March 2023. (B) Distribution of COVID-19 cases among individuals aged 25–29, 40–44, and 70 + years from April 2022 to March 2023. (C) Distribution of COVID-19 cases among individuals aged 25–29, 40–44, and 70 + years from April 2023 to October 2023. Individuals who tested positive for SARS-CoV-2 before March 19, 2023, were considered notifiable. As of March 20, mild cases of COVID-19 were exempt from reporting and isolation requirements, with only patients meeting the criteria for moderate and severe COVID-19 being notifiable.

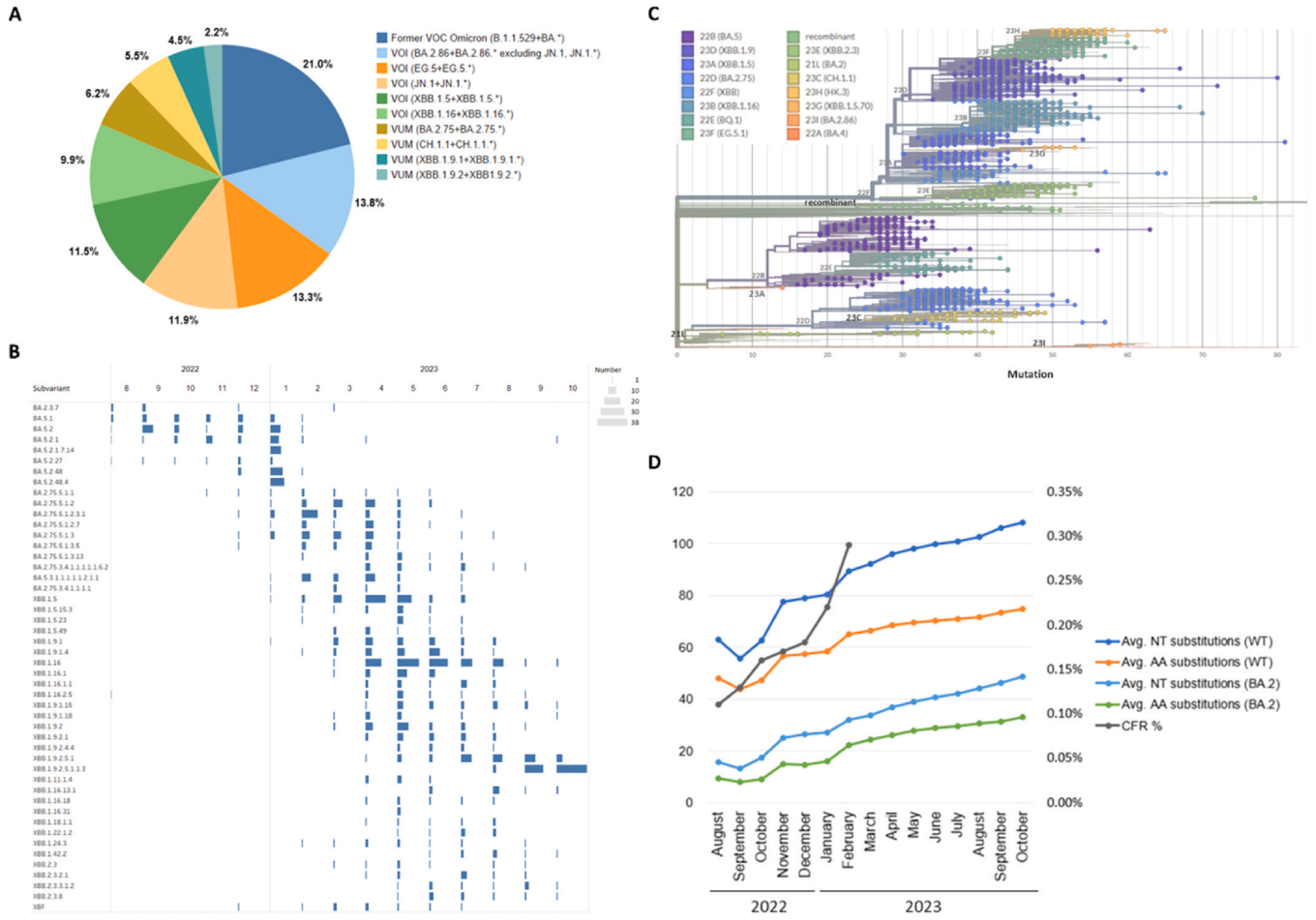

Fig. 3. SARS-CoV-2 sequences identified in Taiwan between August 2022 and October 2023. (A) Omicron subvariant distribution represented in a pie chart. Only subvariants that were identified and present in > 2 % of the population are shown (n = 1919). Variant of concern (VOC), variant of interest (VOI), or variant under monitoring (VUM) designations are shown. (B) Monthly distribution of Omicron subvariants. Only subvariants with a total count exceeding 3 are shown. Please refer to Supplementary Table 3 for additional information (n = 1930). The legend is shown in the upper right. (C) A partial tree derived from placement on a global SARS-CoV-2 phylogenetic tree. All 1930 SARS-CoV-2 sequences identified in Taiwan are shown. (D) Nucleotide and amino acid diversity of the 1930 SARS-CoV-2 sequences identified in Taiwan determined using either wild-type SARS-CoV-2 (Wuhan-Hu-1/2019, MN908947) or the BA.2 subvariant as reference sequences.

that patients with Omicron infections have lower rates of hospitalization, ICU admission, and fatality than do those with previous variants, with the severity order being BA.2 ≤ BA.1 < Delta < Alpha [26–28]. Additionally, numerous countries have adopted policies for coexisting with COVID-19. Taiwan aimed to "eliminate severe cases and control mild cases," shifting to coexistence in March 2022. The average CFR from April 2022 to February 2023, which was in the Omicron era, was 0.18 %, which was lower than that of earlier outbreaks (wild-type 0.80 %, Alpha 5.95 %, Delta 0.94 %) [17,19]. Between January 2020 and March 2022, Taiwan had 24,568 COVID-19 cases in total. From April 2022 to March 2023, there were approximately 851 thousand COVID-19 cases per month. In recent years, amid the global COVID-19 pandemic, Taiwan's monthly COVID-19-related CFR has typically remained below the COVID-19-related CFR worldwide (Fig. 5), except during the Alpha variant-driven COVID-19 outbreak from May to June 2021 [15,17,19]. Changes in the definition of confirmed COVID-19 cases resulted in a significant increase in the monthly CFR from April 2023 onward. Notably, these confirmed cases under the new definition were previously classified as moderate or severe COVID-19. Furthermore, regardless of the diagnostic definition of COVID-19, Taiwan's overall CFR from April 2022 to October 2023 was slightly lower than the global CFR (0.21 % vs. 0.27 %). During the same period, the CFRs in other countries were 0.22 % in Germany and Israel, 0.17 % in Japan, 0.04 % in Singapore, 0.09 % in South Korea, 1.03 % in the United Kingdom, and 0.63 % in the United States (https://ourworldindata.org/). Differences in CFR between countries may be due to factors such as demographics, aging populations, control measures, movement restrictions, and income levels [29,30].

A few months after COVID-19 became a global pandemic in March 2020, several phase III clinical trials of COVID-19 vaccines showed promising results [31]. However, concerns emerged regarding these vaccines—such as their focus on mild cases rather than severe disease, effectiveness in preventing transmission, and safety and potential side effects—sparking some controversy [32]. Most participants in the clinical trials are young and healthy, and the results cannot be generalized to some vulnerable groups, such as children, pregnant women, elderly individuals, immunocompromised patients, patients with coexisting conditions, and patients with severe COVID-19 [33–35]. Taiwan's Ministry of Health and Welfare approved a COVID-19 vaccine (AstraZeneca) for emergency use and the vaccination program that started in March 2021 was initially difficult due to public doubts about the effectiveness and safety of the vaccine. The outbreak caused by the Alpha variant, which started in late April and expanded in May 2021, increased people's willingness to get vaccinated. This may have led to a rapid decline in the CFR despite a sharp increase in the number of COVID-19 cases after the Omicron BA.2.3.7 outbreak in April 2022 (Fig. 4). However, during the subsequent three waves of COVID-19, the vaccination rate had already plateaued. Other research findings

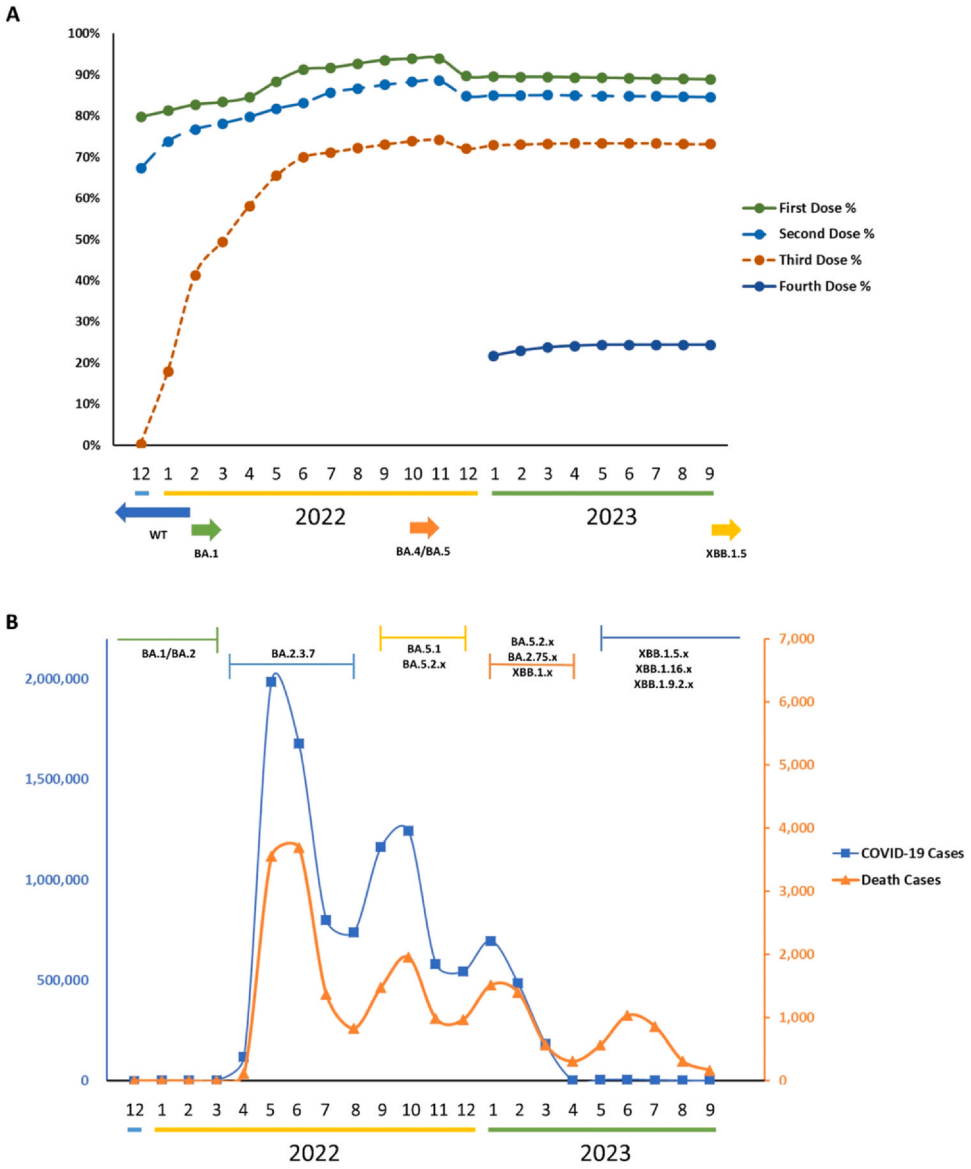

Fig. 4. Vaccination and COVID-19 status in Taiwan between December 2021 and September 2023. (A) Vaccination status (line color according to the legend) and types of COVID-19 vaccines (colored arrows) administered in Taiwan. (B) Monthly COVID-19 cases and COVID-19-related deaths, and the prevalence of predominant SARS-CoV-2 subvariants. Individuals who tested positive for SARS-CoV-2 before March 19, 2023, were classified as notifiable. Starting on March 20, individuals with mild cases of COVID-19 were exempt from reporting and isolation mandates, with only patients meeting the criteria for moderate and severe cases being classified as notifiable.

have indicated that protection against SARS-CoV-2 infection wanes over time after vaccination [34,35]. In addition to vaccination programs, people's acceptance of and preference for vaccines may also play a role in evaluating the effectiveness of COVID-19 vaccines, for which there is insufficient data [36–38]. Thus, we cannot draw conclusions from these limited data whether the decreases in COVID-19 cases, deaths, and CFR are related to the implementation of the vaccination program. More studies are needed to answer this question. The results of a multicenter study in 2023 highlighted the potential benefits of implementing a mixed-and-matched vaccination approach, which could provide varying degrees of risk reduction against infections among healthcare workers, particularly amidst the prevalence of the SARS-CoV-2 Omicron variant [39]. In a comprehensive evaluation of COVID-19 policies and outcomes in 50 countries and territories, Tsao et. al. suggested that effective control of COVID-19 is related to health system policies, local lockdown measures, and vaccine distribution efforts [29]. In a very recent study, Kim et. al. compared the strategies and outcomes used by two different countries, South Korea and the United States, to respond to the COVID-19 pandemic. They reported that the fatality rate in South Korea was approximately five times lower than that in the United States. They concluded that the most important lesson from South Korea's response to COVID-19 was the implementation of rigorous testing and contact tracing, combined with timely information dissemination and vaccine distribution [40]. Taken together, preventive measures and policies other than vaccination also greatly affected the scale of the COVID-19 outbreak.

The ongoing fluctuations in the number of new COVID-19 cases may be linked to the continual replacement of Omicron subvariants, as noted for the first wave (BA.2.3.7 from April 2022 to August 2022) [15], the second wave (BA.5.1 and BA.5.2.x from September 2022 to December 2022), the third wave (BA.5.2.x, BA.2.75.x and XBB.1.x from January 2022 to April 2023), and the fourth wave (XBB.1.5.x, XBB.1.16.x, and XBB.1.9.2.x from May to September 2023) (Fig. 4B and Table 2). In addition, a new COVID-19 wave arose between January and March 2024. However, which variants are causing this latest COVID-19 wave has yet to be determined. Notably, we observed a continued decline in the number of COVID-19 cases and deaths

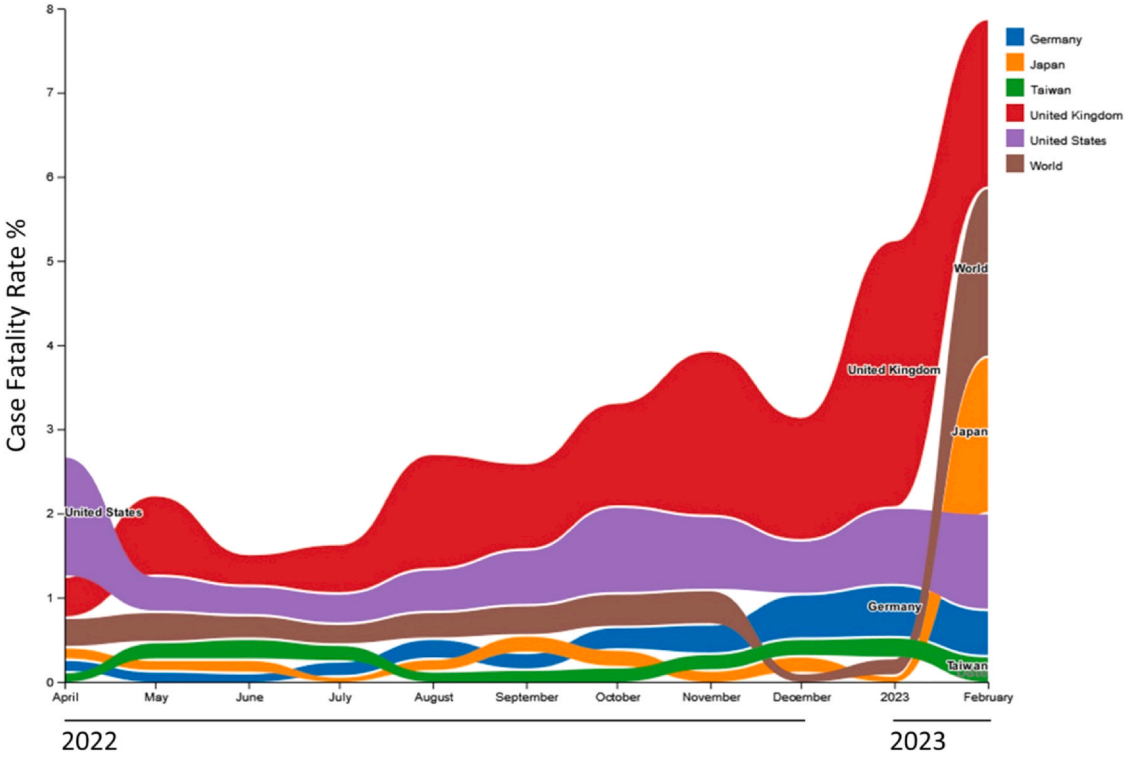

Fig. 5. Case fatality rates in Taiwan, the world and other countries from April 2022 to February 2023.

(Fig. 4B). This may be attributed to COVID-19 vaccination, previous natural infection, cross-reactivity, and hybrid immunity [41,42]. In addition, we noted a rapid increase in the number of monthly Omicron subvariant species from 10 species in August 2022 to 110 species in May 2023 (Supplementary Table 3). This trend suggests that SARS-CoV-2 Omicron may be facing significant evolutionary pressure to survive in nature and under booster vaccine administration [43].

We noted that the confirmed cases between April and October 2023, which encompassed all moderate and severe cases, primarily affected individuals over 70 years old (69.2 %) (Fig. 2C). This trend is consistent with previous research results showing that most moderate and severe cases from January 2022 to January 2023 involved people older than 70 years [15]. We also noted that the vaccination rates for first boosters varied among three different age groups (50–64, 65–74, and > 75 years old) from March 2022 to September 2023, ranging from 58.6 % to 79.8 %, 67.9 % to 81.6 %, and 54.1 % to 72.6 %, respectively. This observation suggested that lower vaccination willingness among older age groups may contribute to the higher fatality rate observed in elderly individuals. Moreover, among those who died from COVID-19, an average of 53–73 % had received fewer than 3 doses of the vaccine, and 74–99 % had at least one chronic medical condition [25]. Huang et al. revealed that "subjects with more comorbidities were less responsive to 3-doses of COVID-19 vaccination serologically" [44]. These results may explain our observations that comorbidity was a risk factor for COVID-19 hospitalization, although the sample size in our study was limited. A recent study by Molaeipour et. al. revealed a correlation between plasma antibody levels in patients with severe COVID-19 and both age and vaccination status. These results underscore the importance of closely monitoring infections among the elderly population and promoting vaccination efforts [45].

Our findings revealed a correlation between the accumulation of NA and AA substitutions in Omicron subvariants and the increase in confirmed COVID-19 cases and related CFR (Fig. 3D and Table 2). Our observation agreed with the finding of DeGrasse et al. that the CFR "began to rise from the Omicron strain through subvariants BA.2/BA.4, BA.5, and XBB.1.5" [46] and was similar to our previous results that the accumulation of NT/AA substitutions was associated with increased Omicron-related COVID-19 severity, whereas all deaths were from severe cases [15]. Maurya et al. reported a greater mutation count per sample in patients who died than in those who recovered [47]. Mutations linked to ACE2-binding affinity, infectivity, and fatality were identified in the Spike protein, including L18F, F58L, L452R, N501Y, and D614G [47–50]. Furthermore, mutations in other genes have been associated with disease severity (ORF3a: Q57H, RdRP: P323L, N: I292T, ORF6: I33T, and nsp7: L71F) [51] and fatality (A1082S, L1546I, and Q2486K in the orf1ab gene; S194L, R203W, and R259Q in the N gene) [47]. Collectively, these studies indicate that the accumulation of genetic variations in SARS-CoV-2 is linked to greater disease severity and fatality in COVID-19 patients. Furthermore, in addition to viral factors, genetic backgrounds, immune statuses; and environmental, clinical, and social factors influence COVID-19-related fatality [52,53].

The newly emerging Omicron subvariants have developed resistance to antibodies from prior natural infections, vaccination, or therapeutic antibodies (such as Evusheld and Bebtelovimab). Multivalent vaccines, live vaccines, and SARS-CoV-2 vaccines that target proteins beyond the Spike protein may be used as booster vaccines [54–56]. In addition to wearing masks, maintaining regular hand hygiene, following social distancing, genomic surveillance, and community monitoring [12], increasing vaccination rates, providing additional booster doses, and focusing on those most at risk of severe disease remain the safest strategies to curb the spread of new SARS-CoV-2 variants [14,55].

There are several limitations of this study. 1. The limited number of COVID-19 patients included in this study hindered exploration of the impact of different subvariants, antiviral drug usage, and COVID-19 vaccination on disease severity, patient hospitalization, and prognosis. 2. The SARS-CoV-2 sequences generated in this study and downloaded from GISAID may not fully represent all epidemic Omicron variants during the investigated period. 3. The COVID-19

data from the Taiwan CDC and the full-length SARS-CoV-2 genome sequences obtained from GISAID lack sufficient detailed clinical information to support analysis of the effects of different subvariants and vaccinations on COVID-19 severity, fatality, hospitalization, and prognosis.

Conclusions

Analysis of clinical data from our COVID-19 cohort indicated that older patients and those with comorbidities were at greater risk of hospitalization. The COVID-19-related CFR generally increased in tandem with the accumulation of NT and AA substitutions in emerging Omicron subvariants. The CFR increased with age and was generally greater in males than in females.

Ethics approval

The current study was reviewed and approved by the Institutional Review Board of KMUH (approval no. KMUHIRB-E-I-20200013). We obtained written informed consent before sample collection.

CRediT authorship contribution statement

Conceptualization: J.J.T., L.T.L., C.H.C.; Methodology: J.J.T., C.H.C., P.C.L., C.Y.T., L.T.L.; Validation: J.J.T., L.T.L., C.H.C., W.L.C., S.J.H., I.W.C.; Formal analysis: J.J.T.; L.T.L., P.C.L., C.Y.T.; Investigation: J.J.T., P.C.C., S.S.C., W.L.C., S.J.H., I.W.C., L.T.L.; Resources: J.J.T., P.C.C., S.S.C., C.H.C., I.W.C., L.T.L.; Data curation: J.J.T., L.T.L., P.C.L., C.Y.T.; Writing—original draft: J.J.T., L.T.L.; Writing—review and editing: J.J.T., L.T.L.; Visualization: J.J.T., L.T.L.; Supervision: J.J.T., C.H.C., W.L.C., S.J.H., I.W.C., L.T.L.; Funding acquisition: J.J.T., L.T.L.

Data Availability

The datasets presented in this study can be found in online repositories. The accession numbers of the 1930 SARS-CoV-2 sequences used in this study can be accessed at https://doi.org/10.55876/gis8.240121qf.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.102556.

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