Travel Medicine and Infectious Disease 65 (2025) 102852

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

The epidemiology and identification of risk factors associated with severe dengue during the 2023 dengue outbreak in Kaohsiung City, Taiwan

Li-Teh Liu [a], Shi-Ya Huang [b], Chen-Hsuan Lin [b], Chun-Hong Chen [c,d], Ching-Yi Tsai [e], Ping-Chang Lin [e], Jih-Jin Tsai [b,e,f,*]

[a] Department of Medical Laboratory Science and Biotechnology, College of Medical Technology, Chung Hwa University of Medical Technology, Tainan City, Taiwan
[b] School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan
[c] National Mosquito-Borne Diseases Control Research Center, National Health Research Institutes, Miaoli County, Taiwan
[d] National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan
[e] Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan
[f] Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

ARTICLE INFO

Keywords:
Dengue virus
Dengue
Severe dengue
Serotype
Risk factors
Secondary infection
Comorbidity

ABSTRACT

After the previous major dengue fever (DF) outbreaks in 2014 and 2015 in Taiwan, the second-largest DF outbreak re-emerged in 2023. A total of 178 patients with laboratory-confirmed dengue virus (DENV) infection, including 92 DENV-1 and 86 DENV-2 cases, were enrolled in this study conducted during the 2023 dengue outbreak in Kaohsiung City, Taiwan. This study aimed to analyze epidemiological characteristics, clinical severity, and risk factors for severe dengue (SD), as well as the diagnostic implications of the non-structural protein 1 (NS1) antigen rapid test. Patients infected with DENV-2 exhibited significantly older age, higher incidence of secondary infections, diabetes mellitus (DM), hypertension (HT), and longer hospital stays than patients infected with DENV-1. Multivariate analysis revealed that older age (age $\geq$65), secondary dengue infection, DM, and HT were significant independent predictors of SD. Compared with non-SD cases, SD patients were significantly more likely to be older (age $\geq$65), to exhibit a higher incidence of secondary infections and a greater prevalence of chronic diseases, including DM and HT. Notably, dengue-confirmed patients with negative NS1 results had a shorter duration since symptom onset ($p < 0.001$). Our DENV-1 and DENV-2 isolates are related to strains from neighboring Asian countries. Our findings emphasize the important factors of old age, secondary infections, and chronic diseases that contributed to dengue severity. We should meticulously manage these high-risk groups to prevent dengue progression. Screening incoming travelers for DF during the epidemic season will be an important measure to prevent the introduction of DENV into Taiwan.

1. Introduction

Dengue virus (DENV) is an arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. Four distinct serotypes (DENV-1 to DENV-4) are differentiated through serological testing [1]. Initially, DENV was confined to tropical and subtropical regions. However, due to factors such as population growth, global warming, and increased urban-rural and international travel, its impact on humans has significantly escalated over the past fifty years [2]. Infection with DENV in humans can lead to various clinical outcomes. According to the 1997 World Health Organization (WHO) dengue guidelines, DENV infection can result in asymptomatic infection, dengue fever (DF), life-threatening dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) [3]. The updated 2009 WHO guidelines further categorize DENV infection into undifferentiated fever, dengue without warning signs (DF), dengue with warning signs (DFWS), and severe dengue (SD) [4].

Prior to 2023, Taiwan had experienced six major DF outbreaks over the past four decades, dating back to 1981 [5]. Taiwan's two largest dengue outbreaks occurred consecutively in 2014 (DENV-1) and 2015 (DENV-2) (Supplementary Fig. 1) [6]. During the pandemic, reductions in international travel, business activities, tourism, and outdoor exposure due to stringent control measures [7] significantly affected the

* Corresponding author. Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, 100, Tzyou 1st Rd, 807377, Kaohsiung City, Taiwan.
E-mail address: jijits@cc.kmu.edu.tw (J.-J. Tsai).

https://doi.org/10.1016/j.tmaid.2025.102852
Received 13 October 2024; Received in revised form 4 April 2025; Accepted 8 April 2025
Available online 10 April 2025

transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other infectious diseases, including dengue [8]. Notably, the number of imported dengue cases in Taiwan between 2020 and 2022 reached its lowest point in the past two decades (Supplementary Fig. 1). After Taiwan abandoned its zero-COVID strategy in March 2022 [9], these conditions changed, possibly contributing to a resurgence of dengue in 2023 (Supplementary Fig. 1) [10]. Moreover, effective dengue prevention efforts from 2016 to 2019, followed by the shift in attention to COVID-19 after 2020, may have resulted in complacency, which could have contributed to the dengue outbreak in 2023. Kaohsiung City, located south of the Tropic of Cancer in southern Taiwan, has the highest cumulative number of DF cases among all cities in the country (Supplementary Fig. 2) [6]. While imported cases likely triggered the initial outbreaks, subsequent dengue outbreaks in Kaohsiung City were associated with weather factors, such as temperature, humidity, precipitation, and typhoons [11,12].

Despite previous investigations identifying multiple risk factors associated with SD, such as older age, secondary infection, and chronic comorbidities, there remains a lack of up-to-date and comprehensive epidemiological and clinical data derived from recent outbreaks, particularly involving emerging dengue serotypes in Taiwan. Given the evolving epidemiological patterns and potential shifts in clinical presentations, clearly identifying these risk factors in the context of the latest dengue outbreaks is critical for effective public health interventions and clinical management. Thus, this study aimed to clarify epidemiological characteristics, identify key risk factors associated with SD during the 2023 dengue outbreak in Kaohsiung City, and assess the diagnostic implications of the dengue NS1 rapid test.

2. Materials and methods

2.1. Sample collection, ethics statement, and dengue definition

This study was conducted at a single medical center, Kaohsiung Medical University Hospital (KMUH), which serves as a major referral hospital for dengue cases in Kaohsiung City, allowing for comprehensive data collection and clinical characterization during dengue outbreaks. Patients with dengue-like symptoms, defined as fever combined with at least two symptoms of headache, retro-orbital pain, muscle or joint pain, rash, nausea, vomiting, or mild hemorrhagic manifestations, between July and December 2023 were invited to participate. Their serum samples were collected using serum separation tubes (Becton Dickinson, USA). All sera were collected within 0–28 days post symptom onset (PSO), and those collected within 7 days PSO were classified as acute-phase samples. We obtained written informed consent prior to sample collection. This study was approved by the Institutional Review Board of KMUH (KMUHIRB-960195).

According to WHO guidelines, dengue cases were categorized as DF, DFWS, and SD. DF is defined as fever with two of the following symptoms: nausea/vomiting, rash, aches and pains, positive tourniquet test, leukopenia. DFWS cases have additional warning signs like abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and increased hematocrit. SD involves severe plasma leakage, severe bleeding, or severe organ involvement [4]. In some analyses, DFWS and SD were defined as non-typical DF and DF was defined as typical DF.

2.2. Real-time quantitative reverse transcription-polymerase chain reaction (qRT–PCR) and serotype-specific RT–PCR

Real-time qRT–PCR was conducted using the Brilliant II SYBR Green qRT-PCR Low ROX Master Mix system (Agilent Technologies, USA). Each reaction consisted of a 25 μL mixture comprising 5 μL of extracted RNA, 0.25 μM each of forward and reverse primers (for either dengue virus detection or serotyping), 2 × SYBR Green Master Mix, RT/RNase Block Enzyme Mixture, and RNase-free water. Amplifications were carried out on an Mx3000P Real-Time PCR System (Agilent Technologies, USA). For pan-dengue virus detection and molecular serotyping, the primer set used was published elsewhere [5,13,14]. A positive control was defined by a threshold cycle (Ct) value ≤ 30 and a melting temperature (Tm) ≥ 79 °C, whereas a negative control exhibited a Ct ≥ 40 and a Tm < 79 °C. Experimental samples were considered positive if either the Ct value was $\leq$30 or the Tm was $\geq$79 °C.

2.3. Detection of preexisting anti-dengue IgG in acute-phase serum

Anti-DENV IgG in acute-phase sera was detected using the InBios DENV Detect IgG ELISA kit (InBios International, Inc., USA) according to the manufacturer's guidelines. A brief procedure and criteria for interpretation were published in our previous studies [5,15]. Each serum sample was tested in duplicate, with a positive result indicating a recent secondary DENV infection and a negative result indicating a recent primary DENV infection [5,15].

2.4. Detection of DENV non-structural protein 1 (NS1) antigen

The presence of the DENV NS1 antigen in serum samples was assessed using the Bioline Dengue NS1 Antigen Test (Abbott, USA) according to the manufacturer's guidelines. Briefly, 100 μL of serum was added to well S, and the result was read after 15–20 min, in accordance with the instructions provided in the package insert.

2.5. Sequencing of DENV envelope gene and phylogenetic analysis

Genomic region containing DENV envelope gene (1485 bp) was amplified by using primers for DENV-1 (DN1-E-F: CCATCACCCAGAAAGGGATT and DN1-E-R: GCCACTTCCACATTTGAGTTC; 1598 bp) and DENV-2 (DN2-E-F: TACTGACAGCTGTTGCTCCTTC and DN2-E-R: TGTTTTTCCAGCTCACAACGC; 1543 bp). We reconstructed phylogenetic trees to explore the relationship between DENV strains identified in Kaohsiung City and DENV isolates from other laboratories collected in 2014, 2015, 2016, and 2023 in Taiwan, as well as DENV sequences retrieved from other Asian countries via EpiArbo at GISAID (https://www.gisaid.org/) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/). We included DENV-1 genotypes I-VI and DENV-2 genotypes I-V to assign the genotype of the DENV isolated in this study. Theoretical phylogenetic trees were reconstructed using sequences of the envelope gene of DENV and the methods from our previous studies [5,9,16–18].

2.6. Clinical data collection and dengue case definitions

Demographic data and information regarding chronic diseases were collected from electronic medical records. DF cases were defined by dengue-like illness with a positive DENV qRT–PCR result; cases with negative results were excluded. SD was identified by one of the following: severe plasma leakage causing shock, fluid accumulation with respiratory distress, severe bleeding, or severe organ impairment (e.g., liver AST/ALT $\geq$1,000, altered consciousness, or cardiac involvement) [4].

2.7. Retrieval of dengue data in Taiwan

Epidemiological dengue data in Taiwan were retrieved from the web-based notifiable diseases surveillance system maintained by the Taiwan Centers for Disease Control (TCDC) (https://nidss.cdc.gov.tw/en/Home/Index) [6]. This data are publicly available. The TCDC classified the cases as either imported or resulting from local transmission.

2.8. PCR amplification and Sanger sequencing

Total RNA was reverse transcribed using HiScript III 1st Strand cDNA Synthesis Kit with random primers (Vazyme Biotech, China) to generate cDNA. For rapid sequencing, amplifications were performed using 10 ng of cDNA with the Phanta Flash PCR Master Mix (Vazyme Biotech, China) and specific target primer pairs for the sequence of the product envelope protein E of dengue virus (DN1-E-F: CCA TCA CCC AGA AAG GGA TT, DN1-E-R: GCC ACT TCC ACA TTT GAG TTC and DN2-E-F: TAC TGA CAG CTG TTG CTC CTT C, DN2-E-R: TGT TTT TCC AGC TCA CAA CGC) at a working concentration of 250 nM on Applied Biosystems 9700 Thermal Cycler (Applied Biosystems, USA) according to the manufacturer's instructions. The thermal cycling program was as follows: 98 °C for 30 s, 40 cycles of 98 °C for 10 s, 60 °C for 5 s, 72 °C for 7 s, with a final extension at 72 °C for 1 min. Amplified products were purified with VAHTS DNA Clean Beads (Vazyme Biotech, China) and analyzed on MultiNA MCE-202 with DNA 2500 Kit (Shimadzu, Japan) to check the target amplicon length and quantity. The purified products were then sequenced with BigDye Terminator v3.1 (Applied Biosystems, USA) bidirectionally by ABI 3730 Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's protocol. Finally, the sequence data were analyzed using Sequencing Analysis Software v5.2 (Applied Biosystems, USA).

2.9. Reconstruction of phylogenetic tree

In brief, envelope gene sequences were aligned with MAFFT v7.526, the best evolutionary model was evaluated, and phylogenetic trees were reconstructed with 1000 bootstrap replicates using IQ-TREE v2.3.5. The theoretical tree is displayed using iTOL version 6.9.1, showing bootstrap values and a scale bar.

2.10. Statistical analysis

Statistical analyses were conducted using SPSS Statistics version 20.0 (IBM Corp., USA). The independent samples *t*-test (two-tailed) was used to analyze age, days PSO, and Charlson comorbidity index score (CCIS) —a tool for predicting long-term mortality [19] —between groups. The Chi-squared test was employed to identify significant differences in categorical variables. For categorical variables with expected frequencies less than 5, Fisher's exact test was applied instead of the Chi-squared test. Multivariate logistic regression was used to estimate odds ratios (ORs) and 95 % confidence intervals (CIs) for variables potentially associated with severe dengue. Variables were selected for inclusion in the multivariate model based on clinical relevance and/or a p-value < 0.15 in univariate analysis. This approach allows identification of independent risk factors while accounting for potential confounders, even in cases where individual associations were not significant in univariate testing due to possible suppression or interaction effects.

3. Results

3.1. Dengue outbreak situations in Taiwan in 2023

Following DF outbreaks in 2014 and 2015, Taiwan experienced its second-largest dengue outbreak since national surveillance began 45 years ago in 2023 [5]. This outbreak began in June 2023 and peaked in September 2023. Similar to previous outbreaks, it started with imported cases and rapidly escalated into autochthonous cases as summer progressed [20,21]. As winter approached, the number of confirmed cases declined (Supplementary Fig. 3). A total of 26,429 autochthonous confirmed DF cases were reported during this outbreak, including 60 deaths, resulting in a case fatality rate of 0.23 % [6]. Most cases occurred in southern Taiwan, particularly in Tainan City and Kaohsiung City, which reported 21,513 and 3145 cases, respectively, accounting for 81.4 % and 11.9 % of all annual cases (Supplementary Fig. 2G). The cumulative number of dengue cases in these two cities over the past 45 years ranks them among the top two of 22 cities in Taiwan [5].

3.2. DENV-1 and DENV-2 were nearly equally distributed in Kaohsiung City during the DF outbreak between July and December 2023

We collected serum samples from patients suspected of having dengue at KMUH, Kaohsiung City, between July 2023 and December 2023. A total of 1368 single acute-phase serum samples were collected, with 183 (13.4 %) testing positive for DENV qRT−PCR and 1185 (86.6 %) testing negative (Fig. 1). The results of serotype-specific RT−PCR revealed that, except for one DENV-3 and four unidentified samples (Fig. 1), DF patients were infected with DENV-1 (n = 92) and DENV-2 (n = 86) at a nearly 1:1 monthly ratio (Fig. 2). The monthly trend in the number of DENV-positive specimens we collected paralleled the confirmed dengue cases in Kaohsiung City (Fig. 2 and Supplementary Fig. 4).

3.3. The differences between DENV-1 and DENV-2-infected cases

There was no difference in disease severity between the patients infected with DENV-1 and those with DENV-2 (Table 1). However, DENV-2-infected cases exhibited a significantly longer length of stay (LOS) in the hospital ($p = 0.028$). Multivariate logistic regression analysis revealed that, compared with DENV-1-infected patients, DENV-2-infected patients were significantly older (age ≥65), had a higher rate of secondary infections, and presented with more chronic diseases, including diabetes mellitus (DM) and hypertension (HT).

3.4. Analysis of age factor in dengue patients

We compared patients aged ≥65 years with those under 65 years (Table 2). The aged patients had a significantly higher rate of DENV-2 infection, greater disease severity, a higher incidence of secondary infections, and an increased prevalence of chronic diseases, including DM, HT, and moderate to severe chronic kidney disease (CKD). Furthermore, older patients had a significantly longer LOS ($p = 0.018$).

3.5. Analysis of the risk factors for severe dengue and non-severe dengue

There was no difference in LOS between SD and non-SD patients (Supplementary Table 1). Compared with non-SD cases, SD patients were significantly older, had a higher incidence of secondary infections and chronic diseases, including DM and HT, highlighting these factors as potential risk indicators for disease severity.

3.6. Analysis of the risk factors for typical dengue and non-typical dengue patients

We analyzed patients with typical DF and those with non-typical DF, including DFWS and SD. Non-typical DF cases exhibited significantly higher CCIS ($p = 0.03$), longer LOS ($p = 0.021$), and a higher prevalence of DM (Supplementary Table 2). A sub-analysis of DENV-1-infected patients suggested no differences between typical DF and non-typical DF cases in all parameters compared (data not shown). In DENV-2-infected patients, non-typical DF cases had significantly higher CCIS and a higher prevalence of DM (Supplementary Table 3).

3.7. Analysis of the factors contributing to the dengue patients with negative NS1 results

The NS1 antigen rapid test was utilized for early dengue detection due to its high sensitivity during the initial phase of infection, enabling prompt diagnosis, appropriate clinical triage, and timely patient management. However, we identified some dengue-confirmed cases with

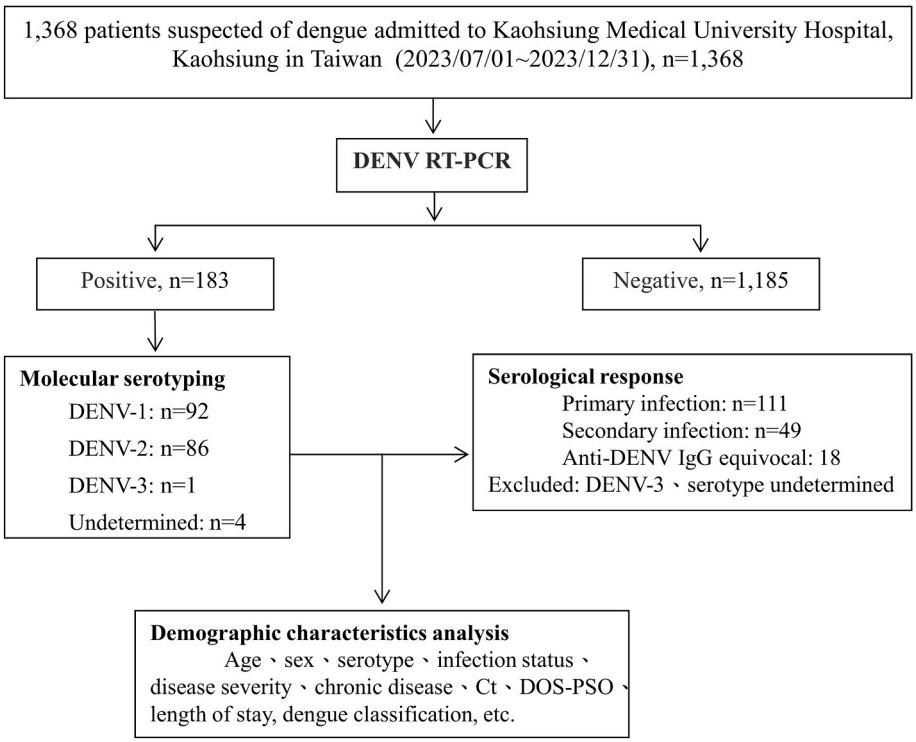


Fig. 1. Flow diagram for recruitment and assessment of study patients. Abbreviations: Ct, Cycle threshold values for DENV in real-time qRT−PCR. DOS, day of sampling. PSO, days post symptom onset.

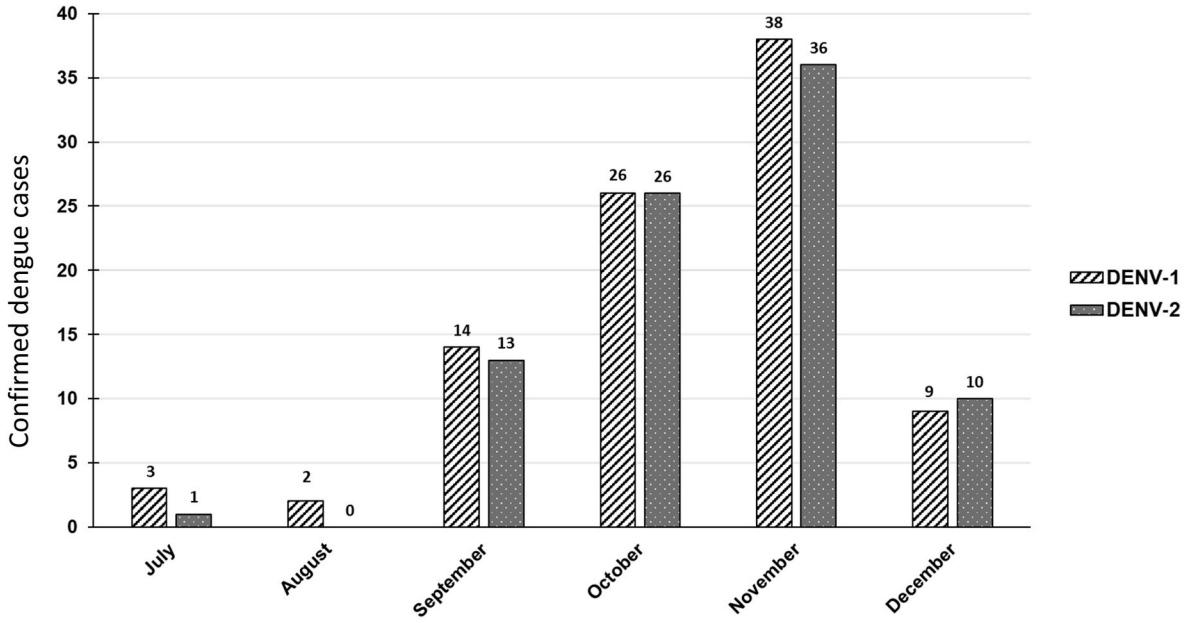


Fig. 2. Monthly distribution of DF patients infected with DENV-1 or DENV-2 between July 2023 and December 2023 in Kaohsiung City in this study.

negative NS1 results; thus, we further analyzed factors associated with these negative outcomes. Comparison between patients with negative NS1 results and those with positive ones showed no differences in DENV-1/DENV-2 infections, primary/secondary infections, Ct (Cycle threshold) values, disease severity, or chronic diseases (Supplementary Table 4). Notably, dengue patients with negative NS1 exhibited significantly shorter duration since symptom onset ($p < 0.001$). The NS1 positivity rates for patients with days PSO ≤3 days and >3 days were 84.3 % (119/141) and 97.3 % (36/37), respectively ($p = 0.05$). However, multivariate analysis suggested that there was no significance between the two groups ($p = 0.068$).

3.8. Sanger sequencing of envelope gene of DENV and phylogenetic analysis

RT−PCR products containing envelope gene sequences of DENV-1 and DENV-2 isolated in this study were subjected to Sanger sequencing and uploaded to GenBank (Supplementary Table 5). We reconstructed phylogenetic trees to explore the potential origins of the DENV circulating in Taiwan during the 2023 dengue outbreak, as

Table 1
Analysis of patients infected with DENV-1 and DENV-2.[a]

Demographic Characteristics[b]	DENV-1	DENV-2	*p*-value	Logistic regression	
	n = 92	N = 86		*p*-value	OR (95 % CI)
Age (Years)					
< 65	69 (75)	46 (53.5)	0.003	0.003	
≥ 65	23 (25)	40 (46.5)			2.61 (1.38–4.92)
Sex					
Male	48 (52.2)	41 (47.7)	ns		
Female	44 (47.8)	45 (52.3)			
Disease severity					
Non-SD	90 (97.8)	80 (93)	0.12	ns	
SD	2 (2.2)	6 (7)			
2009 WHO Dengue Classification					
DF	82 (89.1)	71 (82.6)	ns		
DFWS	8 (8.7)	9 (10.5)			
SD	2 (2.2)	6 (7)			
Infection status[c]					
Primary	63 (76.8)	48 (61.5)	0.036	0.037	
Secondary	19 (23.2)	30 (38.5)			2.07 (1.04–4.12)
Chronic disease					
Charlson comorbidity index score	0 (0–6)	1 (0–5)	0.074		
Diabetes mellitus	12 (13)	23 (26.7)	0.022	0.024	2.43 (1.13–5.27)
Hypertension	19 (20.7)	33 (38.4)	0.009	0.01	2.39 (1.30–4.63)
Congestive heart failure	1 (1)	1 (1)	ns		
COPD	0 (0)	5 (5.8)	0.025		
Cerebrovascular disease	1 (1)	3 (3.5)	ns		
Moderate to severe CKD[d]	9 (9.8)	4 (4.7)	ns		
Malignancy	3 (3.3)	7 (8.1)	ns		
Hyperlipidemia	0 (0)	1 (1.2)	ns		
Length of stay (days)	4 (0–23)	6 (0–34)	0.028		

Note: DENV, dengue virus. Non-SD, Non-severe dengue. SD, Severe dengue. OR, Odds ratio. CI, Confidence interval. DF, dengue fever. DFWS, dengue with warning signs. COPD, Chronic obstructive pulmonary disease. CKD, Chronic kidney disease. ns, non-significant.

[a] The Chi-squared test was employed to identify significant differences in categorical variables in the univariate analyses. For categorical variables with expected frequencies less than 5, Fisher's exact test was applied instead of the Chi-squared test.

[b] Presented as the numbers (%) except for Charlson comorbidity index score and length of stay, which are shown as the median (range).

[c] Samples with equivocal InBios IgG ELISA results were excluded from this analysis.

[d] Moderate CKD: stage 3A & 3B (GFR 45–59 ml/min & 30–44 ml/min), Severe CKD: stage 4 & 5 (GFR 15–29 ml/min & less than 15 ml/min).

detailed in the Materials and Methods section. The results indicate that DENV-1 isolates from this study, collected between July and November 2023, were closest to Taiwan/717 TN2306a, the first indicative DENV-1 isolate in 2023, which was collected by TCDC in June 2023 in Tainan City—approximately 50 km north of Kaohsiung City. This suggests that DENV-1 circulating in Kaohsiung City may have been transmitted from Tainan rather than being directly imported from other Asian countries (Fig. 3A and Supplementary Fig. 2G). This DENV-1 genotype I lineage K.1 outbreak may have spread from Tainan City to Kaohsiung City between July and August. The I.K.1 DENV-1 was likely imported from Singapore or Japan, as our DENV-1 isolates were phylogenetically close to the Singapore/EHI-20950Y23/2023 and the Japanese isolates (Japan/TMIPH-23008/2023 and Japan/TMIPH-23010/2023), which were isolated on May 15, 2023, in Singapore and on June 1, 2023, in Japan, prior to the outbreak's commencement in Tainan City (Fig. 3A and Supplementary Fig. 4).

Further results suggested that DENV-2 isolates collected between September and December 2023 in this study were closest to Taiwan/813 KH2307a, which was the first indicative DENV-2 isolate in 2023 identified by TCDC in July 2023 in Kaohsiung City among all DENV-2 sequences isolated in Taiwan (Fig. 3B). This genotype II lineage F.1 DENV-2 was likely imported from Vietnam or Malaysia to Kaohsiung City, as our DENV-2 isolates were phylogenetically close to the Vietnam/Yale-02307/2023 and the Malaysian isolates (Malaysia/TIDREC_KLA-AUFI042/2023, Malaysia/TIDREC_KJA-AUFI064/2023, and Malaysia/TIDREC_KJA-AUFI052/2023), which were isolated on February 2, 2023, in Vietnam and in June 2023 in Malaysia, prior to the outbreak's commencement in Kaohsiung City (Fig. 3B and Supplementary Fig. 4).

4. Discussion

Dengue fever has long posed a significant public health concern in Kaohsiung City. Our study revealed that the ratio of DENV-1 to DENV-2 in Kaohsiung is nearly 1:1. However, this may not reflect the overall situation of the 2023 DENV outbreak in Taiwan. Data released by the TCDC indicated that 2989 autochthonous DENV-1 cases and 1002 DENV-2 cases were identified across Taiwan in 2023. Additionally, only ten DENV-3 and seven DENV-4 cases were detected among imported dengue cases. Other cases were either undetermined or not identified in the serotype surveillance [6]. This suggests that, for unclear reason(s), DENV-3 and DENV-4 were not involved in the 2023 dengue outbreak. The dengue outbreak in 2023 began in Tainan (Supplementary Fig. 4) with the first identified serotype being DENV-1 (Fig. 3A). The subsequent dengue outbreak in Kaohsiung City may have been caused by both DENV-1 and DENV-2 (Fig. 2). These findings might be related to the serotypes that previously circulated in these two cities. In 2014, Taiwan reported a total of 15,492 dengue cases, with DENV-1 as the predominant serotype [22,23]. Of these, 96.8 % occurred in Kaohsiung City (n = 14,999), while only 1.0 % was reported in Tainan City (n = 156).

In 2015, Taiwan recorded 43,419 dengue cases, with DENV-2 as the predominant serotype. Of these, 52.4 % of the cases occurred in Tainan City (n = 22,760) and 45.4 % in Kaohsiung City (n = 19,723) [6]. Our previous study indicated that 93.7 % of the DENV identified in Kaohsiung City in 2015 were DENV-2 (1550/1655), with only 6.3 % of DENV-1 (105/1655) [5]. Given the data from these two major dengue outbreaks in Taiwan, the ratio of DENV-1 to DENV-2 in Kaohsiung City and Tainan City was approximately 43.2 %–56.8 % and 0.7 %–99.3 %, respectively. This suggests that the seroprevalence of DENV-1 and DENV-2 among residents of Kaohsiung may be roughly proportional at 3:4, while residents of Tainan may have relatively lower immunity to

Table 2
Analysis of age factor in dengue patients.[a]

Demographic Characteristics[b]	<65 yo	≥65 yo	p-value	Logistic regression	
	n = 115	n = 63		p-value	OR (95 % CI)
Sex					
Male	63 (54.8)	26 (41.3)	0.085	ns	
Female	52 (45.2)	37 (58.7)			
Serotype					
DENV-1	69 (60)	23 (36.5)	0.003	0.003	
DENV-2	46 (40)	40 (63.5)			2.61 (1.38–4.92)
Disease severity					
Non-SD	113 (98.3)	57 (90.5)	0.024	0.032	
SD	2 (1.7)	6 (9.5)			5.95 (1.16–30.41)
2009 WHO Dengue Classification					
DF	101 (87.8)	52 (82.5)	0.053		
DFWS	12 (10.4)	5 (8)			
SD	2 (1.7)	6 (9.5)			
Infection status[c]					
Primary	88 (84.6)	23 (41.1)	<0.001	<0.001	
Secondary	16 (15.4)	33 (58.9)			7.89 (3.72–16.76)
Chronic disease					
Charlson comorbidity index score	0 (0–4)	2 (0–6)	<0.001		
Diabetes mellitus	8 (7)	27 (42.9)	0.127	<0.001	10.03 (4.18–24.06)
Hypertension	12 (10.4)	40 (63.5)	0.043	<0.001	14.93 (6.79–32.82)
Congestive heart failure	1 (0.9)	1 (1.6)	ns		
COPD	1 (0.9)	4 (6.3)	ns		
Cerebrovascular disease	0	4 (6.3)	0.015		
Moderate to severe CKD[d]	4 (3.5)	9 (14.3)	0.013	0.014	4.63 (1.36–15.70)
Malignancy	4 (3.5)	6 (9.5)	ns		
Hyperlipidemia	0 (0)	1 (1.6)	ns		
Length of stay (days)	5 (0–34)	6 (0–23)	0.018		

Note: yo, years old. DENV, dengue virus. Non-SD, Non-severe dengue. SD, Severe dengue. OR, Odds ratio. CI, Confidence interval. DF, dengue fever. DFWS, dengue with warning signs. COPD, Chronic obstructive pulmonary disease. CKD, Chronic kidney disease. ns, non-significant.

[a] The Chi-squared test was employed to identify significant differences in categorical variables in the univariate analyses. For categorical variables with expected frequencies less than 5, Fisher's exact test was applied instead of the Chi-squared test.

[b] Presented as the numbers (%) except for Charlson comorbidity index score and length of stay, which are shown as the median (range).

[c] Samples with equivocal InBios IgG ELISA results were excluded from this analysis.

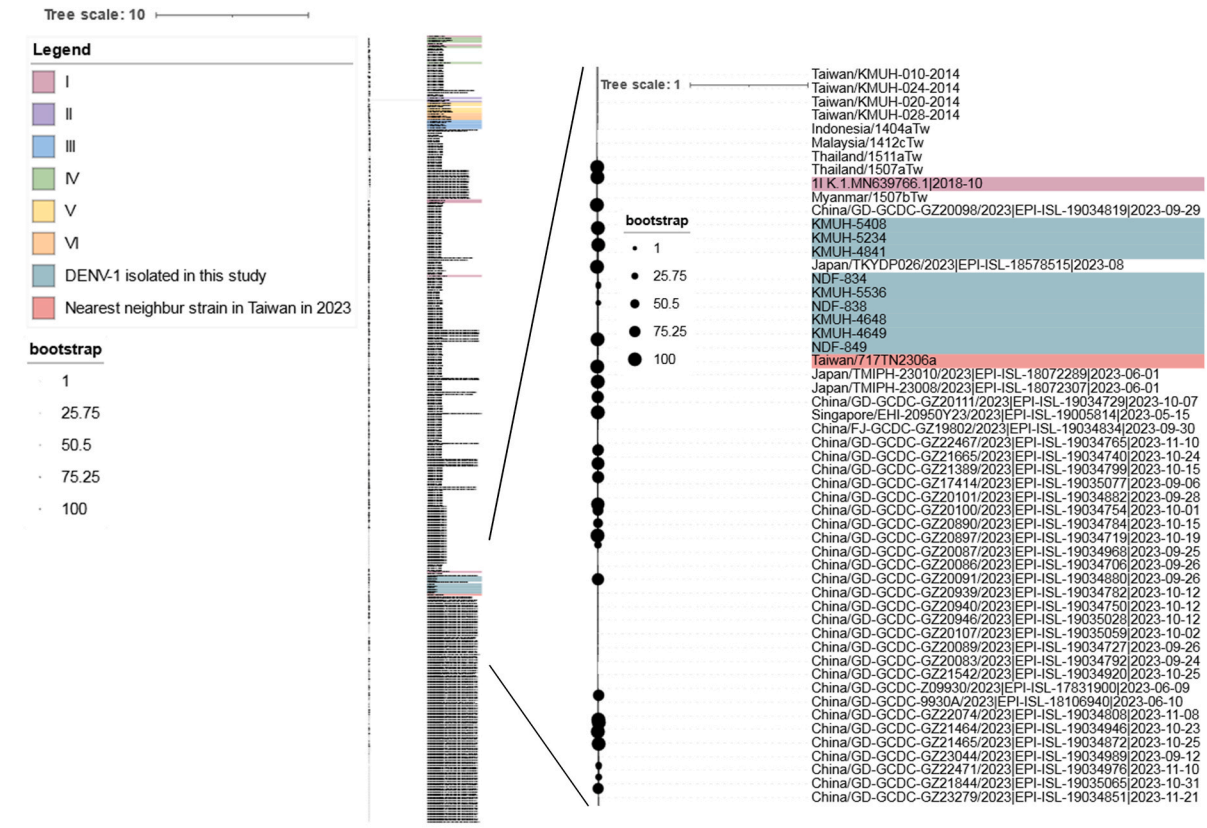
[d] Moderate CKD: stage 3A & 3B (GFR 45–59 ml/min & 30–44 ml/min), Severe CKD: stage 4 & 5 (GFR 15–29 ml/min & less than 15 ml/min).

DENV-1.

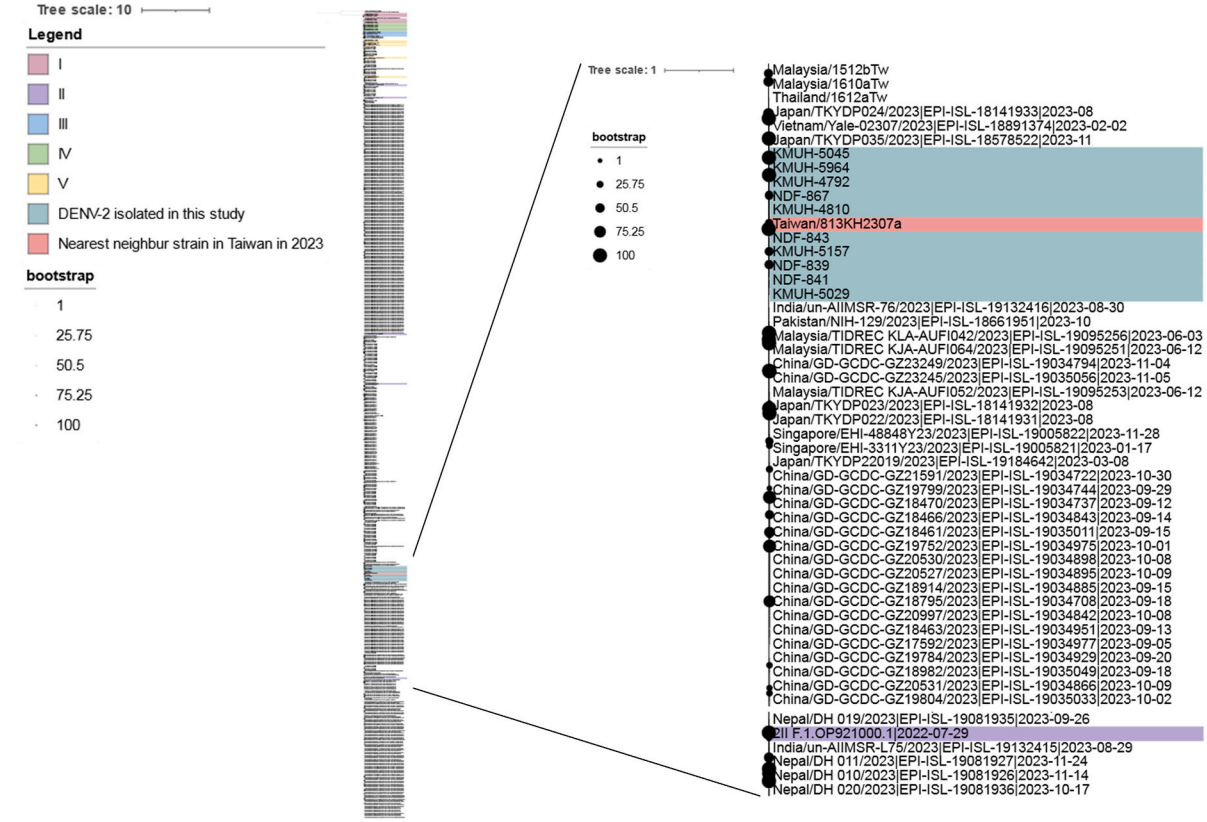
Serotype surveillance data from the TCDC in 2023 showed 1720 DENV-1 and 974 DENV-2 cases in Kaohsiung, compared to 1027 DENV-1 and 31 DENV-2 cases in Tainan. This clearly indicates that Tainan residents have relatively lower herd immunity to DENV-1. Another possibility is that DENV-2 was detected in Tainan only in October, when the outbreak was gradually subsiding. Notably, DENV-1 was identified in autochthonous cases in Tainan in early June, while local cases in Kaohsiung City emerged in late June. DENV-1 in Kaohsiung may have been transmitted from Tainan (Fig. 3A and Supplementary Fig. 5). Additionally, DENV-2 appeared in Kaohsiung City as early as July. Although cocirculation of multiple DENV serotypes increases the risk of simultaneous infection with more than one serotype and is associated with SD [24], we did not observe any cases of coinfection with multiple serotypes in this study.

Previous studies have reported inconsistent findings regarding the severity differences between DENV-1 and DENV-2 infections [25,26]. In this study, after properly accounting for age, secondary infection status, and comorbidities using multivariate logistic regression analysis, we observed no significant difference in dengue severity between DENV-1 and DENV-2 infections, which aligns with previous findings in Taiwan [5,27]. This indicates that factors other than serotype itself, such as age and underlying chronic conditions, might play a more critical role in determining disease severity. We further strengthened our analysis by adjusting for potential confounders, including age ≥65 years, secondary infection, DM, and HT. After these adjustments, older age, secondary infection, DM and HT remained independent and significant predictors for SD, reinforcing the necessity for targeted clinical monitoring and proactive management in these high-risk groups. These results are further supported by recent systematic reviews on predictors of SD. Sangkaew et al. identified female gender, older age, DM, HT, renal disease, cardiovascular disease, secondary infection, and DENV-2 infection as important predictors for progression to SD [28]. In addition, Tsheten et al. reported child age, DM, renal disease, and secondary infection as significant risk factors for SD [29]. These analyses underscore that older age, secondary dengue infections, and chronic comorbidities—particularly DM, HT, and renal disease—consistently contribute to increased dengue severity. Thus, our findings support existing evidence and emphasize the importance of identifying and closely monitoring these high-risk groups during dengue outbreaks. In this study, we found that patients infected with DENV-2 were significantly more likely to be aged ≥65 years, have secondary infections, present with chronic diseases such as DM and HT, and experience longer LOS. Similarly, individuals aged ≥65 years were significantly more likely to develop secondary infection, SD, and experience longer LOS than younger patients. Additionally, SD was associated with secondary infection, as supported by recent studies. Shih et al. revealed that "secondary dengue infection significantly increases the risk of severe disease in Taiwan" in a population-based cohort study [30], which is consistent with the findings of Rowe et al., which concluded that elderly patients are at a higher risk of developing DHF and SD [31]. Our findings also align with the results of Wang et al., which showed that anti-DENV IgG seroprevalence rates increase with age [15]. Moreover, patients aged ≥65 years were significantly associated with higher CCIS and were more likely to progress to SD. Our results confirmed the findings from a study conducted in Thailand by Huang et al., which showed that DHF cases continue to increase with age and are associated with individuals with more comorbidities [32]. A previous study showed that females are more likely to develop DSS than males [33]. The results of Anders et al. and our previous study suggested that females were at a significantly higher risk of developing DSS [34]. However, the results of this study did not show differences in dengue severity between females and males. Finally, we found that patients who tested negative for NS1 had shorter duration since symptom onset. Our findings aligned with those of Tricou et al., indicating that the NS1 rapid test exhibits relatively lower sensitivity in serum samples collected within 3 days PSO [35]. This may be attributed to patients being in the early stages of dengue virus infection, where NS1 may not have reached detectable levels. In our cohort, 12.9 % (23/178) of dengue-confirmed patients had negative NS1 results, potentially delaying diagnosis and treatment. Our findings also suggest that NS1 status in dengue-confirmed patients was not correlated with DENV serotype, primary or secondary infections, Ct value, severity, or chronic disease status. Previous studies have indicated that a negative NS1 rapid test result in dengue-confirmed cases is associated with

A



B



(caption on next page)

Fig. 3. Phylogenetic tree of DENV-1 and DENV-2 envelope genes derived from clinical samples collected in Kaohsiung City during the 2023 dengue outbreak. (A) A representative phylogenetic tree of 447 DENV-1 envelope gene sequences including 9 DENV-1 isolates from this study, sequences isolated in Asian countries and retrieved from EpiArbo at GISAID, and reference sequences obtained from GenBank. One DENV-2 sequence (DENV-2/Indonesia/1407aTw) was used as an outgroup. (B) A representative phylogenetic tree of 587 DENV-2 envelope gene sequences including 10 DENV-2 isolates from this study, sequences isolated in Asian countries and retrieved from EpiArbo at GISAID and GenBank, and reference sequences obtained from GenBank. One DENV-1 sequence (DENV-1/Malaysia/1412cTw) was used as an outgroup. A virus with the abbreviation TN in its name was isolated from clinical samples collected in Tainan City. A virus with the abbreviation KH was isolated from clinical samples collected in Kaohsiung City. The two letters "Tw" at the end of the virus name indicate that the sequence was imported into Taiwan from the country in the name.

secondary infections and specific dengue virus serotypes [36–39]. However, the findings of this study did not support these associations.

Given the epidemiological view derived from risk factor analysis, exploring effective preventive measures, such as vaccination, becomes particularly relevant for future dengue outbreak preparedness. Recent advancements in dengue vaccines have shown promise in reducing disease severity and hospitalization rates, especially among high-risk populations such as older adults and individuals with previous dengue infections. The CYD-TDV (Dengvaxia) vaccine has been licensed for use in several dengue-endemic countries and is recommended specifically for individuals who have previously experienced dengue infection, to prevent subsequent infections and reduce severity [40,41]. Additional vaccine candidates, including TAK-003 (QDENGA), have demonstrated promising efficacy and safety profiles in recent clinical trials, further highlighting their potential as preventive tools in future dengue control strategies [42,43]. Given our findings of increased SD risks among older patients, secondary infection cases, and those with chronic conditions, vaccination programs prioritizing these vulnerable groups may significantly alleviate dengue's public health burden. Therefore, integrating dengue vaccines into public health policies and targeted vaccination campaigns should be strongly considered as part of a comprehensive strategy to mitigate future dengue outbreaks.

In addition to clinical risk factors influencing dengue severity, understanding the epidemiological dynamics through phylogenetic analysis provides further context for outbreak control and management strategies. Dengue is not native to Taiwan. Outbreaks in Taiwan generally began with imported cases from Southeast Asia, spreading during the rainy and warm months starting in July, and typically peaking between September and November [44]. Over the past twenty years, various DENV serotypes and clades from Southeast Asian countries like the Philippines, Thailand, Vietnam, Malaysia, Indonesia, Singapore, and Cambodia have been introduced into Taiwan, leading to dengue epidemics of varying severity [23,45,46]. Our results suggested that DENV-1 and DENV-2 isolates in this study were phylogenetically close to the DENV strains in neighboring Asian countries. Notably, in 2023, Vietnam and Malaysia were the primary sources of imported dengue cases in Taiwan, accounting for 70 cases (ranked 1st) and 35 cases (ranked 4th), respectively, while Singapore contributed 8 cases (ranked 8th) [6]. These phylogenetic findings have practical implications for future dengue outbreak preparedness and clinical triage protocols. Enhanced epidemiological surveillance and cross-regional cooperation between health authorities in Kaohsiung City and Tainan City, as well as strengthened international surveillance focusing on travelers from dengue-endemic regions such as Vietnam and Malaysia, are essential. Furthermore, these phylogenetic insights can inform clinical triage protocols by alerting healthcare providers to consider recent travel history, particularly from endemic or neighboring areas experiencing dengue activity, thereby facilitating early diagnosis, appropriate isolation measures, and prompt clinical management.

Despite these clinical and epidemiological findings, several limitations of our study must be acknowledged. First, we collected only single serum samples rather than paired sera, which limited our ability to use convalescent sera to detect anti-DENV antibodies. This prevented us from including more potential DF cases and tracking their symptoms, progression, and prognosis. Second, the sample size for both DENV-1 and DENV-2 was small, limiting our ability to analyze differences in clinical severity or outcomes among dengue patients infected with specific serotypes. Third, our study was conducted at a single medical center (KMUH), the results may not be fully generalizable to other healthcare settings or populations. Local epidemiological patterns, patient demographics, and clinical practices specific to our center could differ substantially from other regions or institutions. Future multicenter studies are warranted to confirm and expand upon our findings. Fourth, we used limited sequences and focused on the envelope gene rather than the full-length DENV genome to reconstruct the phylogenetic tree. At the time of our study, the only available sequences from autochthonous cases in Taiwan were the two envelope gene sequences uploaded by the TCDC, as shown in Fig. 3A and B. Caution should be exercised when interpreting phylogenetic proximity results, given the limitations inherent in analyses based on partial genome sequences. Comprehensive genome sequencing and broader sampling are necessary for definitive conclusions regarding viral transmission routes. Additionally, due to the relatively limited sample size for certain comorbid conditions (e.g., congestive heart failure and COPD), caution is warranted when interpreting these specific associations. Future studies with larger cohorts would be beneficial to confirm the robustness of these risk predictors. Lastly, the results of this study do not consider additional factors such as socioeconomic status and patient genetic background.

5. Conclusions

To the best of our knowledge, this is the first report detailing the epidemiology and clinical observations of the 2023 dengue fever outbreak in Taiwan. In our study, there was no significant difference in dengue severity between DENV-1 and DENV-2-infected cases. However, SD patients were significantly older and exhibited a higher prevalence of secondary infections and chronic diseases, including DM and HT. Among dengue-confirmed patients, there were no differences in DENV-1/DENV-2 serotypes, primary/secondary infections, Ct values, disease severity, or chronic disease status between those with positive and negative NS1 results. Notably, dengue patients with negative NS1 had significantly shorter duration since symptom onset. Our DENV-1 and DENV-2 isolates are closely related to strains from neighboring Asian countries. These findings underscore the importance of old age, secondary infections, and chronic conditions—especially DM and HT—as contributing factors to dengue severity. Therefore, meticulous management of these high-risk groups is crucial to preventing dengue progression. Additionally, screening incoming travelers for DF during the epidemic season will be essential to prevent the introduction of DENV into Taiwan. These conclusions are further supported by two recent systematic reviews from Sangkaew et al. [28] and Tsheten et al. [29], which emphasize the crucial role of older age, secondary infections, and comorbidities—particularly DM and HT—as key risk factors for severe dengue.

CRediT authorship contribution statement

Li-Teh Liu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shi-Ya Huang:** Investigation, Formal analysis. **Chen-Hsuan Lin:** Investigation, Formal analysis. **Chun-Hong Chen:** Validation, Supervision, Methodology, Conceptualization. **Ching-Yi Tsai:** Methodology, Formal analysis, Data curation. **Ping-Chang Lin:** Methodology, Formal analysis, Data curation. **Jih-Jin Tsai:** Writing – review & editing, Writing – original draft,

Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The datasets presented in this study can be found in online repositories. Details of the repositories and accession numbers are provided in the Supplementary Materials. Further inquiries can be directed to the corresponding author.

Ethics approval

The current study was reviewed and approved by the Institutional Review Board of KMUH (approval no. KMUHIRB-960195). Written informed consent was obtained from all participants prior to sample collection.

Funding

This work was supported by the National Health Research Institutes, Taiwan (NHRI-110A1-MRCO-03212101, https://www.nhri.edu.tw/eng, to JJT); and Kaohsiung Medical University Hospital, Taiwan (KMUH-DK(A)113005 and S11314, https://www.kmuh.org.tw). The funders had no role in the study design, data collection, analysis, interpretation, report writing, or the decision to submit the article for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We extend our sincere appreciation to all the data contributors, including the authors and their originating laboratories responsible for specimen acquisition, as well as the submitting laboratories for generating the genetic sequences and metadata. This research is built upon data shared via GISAID and GenBank, and we acknowledge their valuable contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2025.102852.

References

[1] Russell PK, Nisalak A. Dengue virus identification by the plaque reduction neutralization test. J Immunol 1967;99(2):291–6.
[2] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496(7446):504–7.
[3] World Health Organization. Dengue Haemorrhagic fever: diagnosis, treatment, prevention and control. second ed. Geneva, Switzerland: World Health Organization; 1997.
[4] World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva, Switzerland: World Health Organization; 2009.
[5] Tsai JJ, Chang K, Chen CH, Liao CL, Chen LJ, Tsai YY, et al. Dengue virus serotype did not contribute to clinical severity or mortality in Taiwan's largest dengue outbreak in 2015. Eur J Med Res 2023;28(1):482.
[6] Taiwan Centers for Disease Control. Taiwan national infectious disease Statistics system. Available from: https://nidss.cdc.gov.tw/en/Home/Index. [Accessed 17 August 2024].
[7] Chiu NC, Chi H, Tai YL, Peng CC, Tseng CY, Chen CC, et al. Impact of wearing masks, hand hygiene, and social distancing on influenza, enterovirus, and all-cause pneumonia during the coronavirus pandemic: retrospective national epidemiological surveillance study. J Med Internet Res 2020;22(8):e21257.
[8] Lin SF, Lai CC, Chao CM, Tang HJ. Impact of COVID-19 preventative measures on dengue infections in Taiwan. J Med Virol 2021;93(7):4063–4.
[9] Liu LT, Chiou SS, Chen PC, Chen CH, Lin PC, Tsai CY, et al. Epidemiology and analysis of SARS-CoV-2 omicron subvariants BA.1 and 2 in taiwan. Sci Rep 2023;13(1):16583.
[10] Chiu NC, Chi H, Weng SL, Lin CY. Unprecedented dengue outbreak in Taiwan following COVID-19. J Trav Med 2024;31(4):taae069.
[11] Chang K, Chen CD, Shih CM, Lee TC, Wu MT, Wu DC, et al. Time-lagging interplay effect and excess risk of meteorological/mosquito parameters and petrochemical gas explosion on dengue incidence. Sci Rep 2016;6:35028.
[12] Kao B, Lin CH, Wen TH. Measuring the effects of typhoon trajectories on dengue outbreaks in tropical regions of Taiwan: 1998-2019. Int J Biometeorol 2023;67(8):1311–22.
[13] Liu LT, Chen CH, Tsai CY, Lin PC, Hsu MC, Huang BY, et al. Evaluation of rapid diagnostic tests to detect dengue virus infections in Taiwan. PLoS One 2020;15(9):e0239710.
[14] Liu LT, Chen CH, Lin PC, Tsai CY, Hsu MC, Huang BY, et al. Evaluation of a new NS1 rapid diagnostic test using a single acute-phase serum panel collected during the largest dengue outbreak in Taiwan history in 2015. Kaohsiung J Med Sci 2022;38(4):385–9.
[15] Tsai JJ, Liu CK, Tsai WY, Liu LT, Tyson J, Tsai CY, et al. Seroprevalence of dengue virus in two districts of Kaohsiung City after the largest dengue outbreak in Taiwan since World War II. PLoS Neglected Trop Dis 2018;12(10):e0006879.
[16] Liu LT, Tsai JJ, Chen CH, Lin PC, Tsai CY, Tsai YY, et al. Isolation and identification of a rare spike gene double-deletion SARS-CoV-2 variant from the patient with high cycle threshold value. Front Med (Lausanne) 2022;8:822633.
[17] Liu LT, Tsai JJ, Chang K, Chen CH, Lin PC, Tsai CY, et al. Identification and analysis of SARS-CoV-2 alpha variants in the largest taiwan COVID-19 outbreak in 2021. Front Med (Lausanne) 2022;9:869818.
[18] Liu LT, Tsai JJ, Chu JJH, Chen CH, Chen LJ, Lin PC, et al. The identification and phylogenetic analysis of SARS-CoV-2 delta variants in Taiwan. Kaohsiung J Med Sci 2023;39(6):624–36.
[19] Charlson ME, Carrozzino D, Guidi J, Patierno C. Charlson comorbidity index: a critical review of clinimetric properties. Psychother Psychosom 2022;91(1):8–35.
[20] Wang SF, Chang K, Lu RW, Wang WH, Chen YH, Chen M, et al. Large Dengue virus type 1 outbreak in Taiwan. Emerg Microb Infect 2015;4(8):e46.
[21] Wang SF, Chang K, Loh EW, Wang WH, Tseng SP, Lu PL, et al. Consecutive large dengue outbreaks in Taiwan in 2014-2015. Emerg Microb Infect 2016;5(12):e123.
[22] Wei HY, Shu PY, Hung MN. Characteristics and risk factors for fatality in patients with dengue hemorrhagic fever, taiwan, 2014. Am J Trop Med Hyg 2016;95(2):322–7.
[23] Yang CF, Chang SF, Hsu TC, Su CL, Wang TC, Lin SH, et al. Molecular characterization and phylogenetic analysis of dengue viruses imported into Taiwan during 2011-2016. PLoS Neglected Trop Dis 2018;12(9):e0006773.
[24] Vaddadi K, Gandikota C, Jain PK, Prasad VSV, Venkataramana M. Co-circulation and co-infections of all dengue virus serotypes in Hyderabad, India 2014. Epidemiol Infect 2017;145(12):2563–74.
[25] Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon IK, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLoS Neglected Trop Dis 2010;4(3):e617.
[26] Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. Am J Trop Med Hyg 2015;92(5):999–1005.
[27] Tsai JJ, Chan KS, Chang JS, Chang K, Lin CC, Huang JH, et al. Effect of serotypes on clinical manifestations of dengue fever in adults. J Microbiol Immunol Infect 2009;42(6):471–8.
[28] Sangkaew S, Ming D, Boonyasiri A, Honeyford K, Kalayanarooj S, Yacoub S, et al. Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. Lancet Infect Dis 2021;21(7):1014–26.
[29] Tsheten T, Clements ACA, Gray DJ, Adhikary RK, Furuya-Kanamori L, Wangdi K. Clinical predictors of severe dengue: a systematic review and meta-analysis. Infect Dis Poverty 2021;10(1):123.
[30] Shih HI, Wang YC, Wang YP, Chi CY, Chien YW. Risk of severe dengue during secondary infection: a population-based cohort study in Taiwan. J Microbiol Immunol Infect 2024;57(5):730–8.
[31] Rowe EK, Leo YS, Wong JG, Thein TL, Gan VC, Lee LK, et al. Challenges in dengue fever in the elderly: atypical presentation and risk of severe dengue and hospital-acquired infection [corrected]. PLoS Neglected Trop Dis 2014;8(4):e2777.
[32] Huang AT, Takahashi S, Salje H, Wang L, Garcia-Carreras B, Anderson K, et al. Assessing the role of multiple mechanisms increasing the age of dengue cases in Thailand. Proc Natl Acad Sci U S A 2022;119(20):e2115790119.
[33] Kabra SK, Jain Y, Pandey RM, Madhulika Singhal T, Tripathi P, et al. Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. Trans R Soc Trop Med Hyg 1999;93(3):294–8.
[34] Anders KL, Nguyet NM, Chau NV, Hung NT, Thuy TT, Lien le B, et al. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg 2011;84(1):127–34.
[35] Tricou V, Vu HT, Quynh NV, Nguyen CV, Tran HT, Farrar J, et al. Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. BMC Infect Dis 2010;10:142.
[36] Osorio L, Ramirez M, Bonelo A, Villar LA, Parra B. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virol J 2010;7:361.
[37] Kassim FM, Izati MN, TgRogayah TA, Apandi YM, Saat Z. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. Southeast Asian J Trop Med Publ Health 2011;42(3):562–9.

[38] Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, et al. Evaluation of a dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute dengue virus infection. PLoS Neglected Trop Dis 2014;8(10):e3193.
[39] Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, et al. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. PLoS One 2014;9(11):e113411.
[40] Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373(13):1195–206.
[41] Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. N Engl J Med 2018;379(4):327–40.
[42] Biswal S, Reynales H, Saez-Llorens X, Lopez P, Borja-Tabora C, Kosalaraksa P, et al. Efficacy of a tetravalent dengue vaccine in healthy children and adolescents. N Engl J Med 2019;381(21):2009–19.
[43] Rivera L, Biswal S, Saez-Llorens X, Reynales H, Lopez-Medina E, Borja-Tabora C, et al. Three-year efficacy and safety of takeda's dengue vaccine candidate (TAK-003). Clin Infect Dis 2022;75(1):107–17.
[44] Tsai JJ, Lin PC, Tsai CY, Wang YH, Liu LT. Low frequency of asymptomatic dengue virus-infected donors in blood donor centers during the largest dengue outbreak in Taiwan. PLoS One 2018;13(10):e0205248.
[45] Shu PY, Su CL, Liao TL, Yang CF, Chang SF, Lin CC, et al. Molecular characterization of dengue viruses imported into Taiwan during 2003-2007: geographic distribution and genotype shift. Am J Trop Med Hyg 2009;80(6):1039–46.
[46] Huang JH, Su CL, Yang CF, Liao TL, Hsu TC, Chang SF, et al. Molecular characterization and phylogenetic analysis of dengue viruses imported into Taiwan during 2008-2010. Am J Trop Med Hyg 2012;87(2):349–58.