Journal of Microbiology, Immunology and Infection 57 (2024) 269—277

Available online at www.sciencedirect.com

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journal homepage: www.e-jmii.com

Original Article

Detection of toxigenic $M1_{UK}$ lineage group A *Streptococcus* clones in Taiwan

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Received 24 August 2023; received in revised form 6 November 2023; accepted 12 January 2024
Available online 20 January 2024

KEYWORDS
Group A *Streptococcus*;
SSA;
$M1_{UK}$;
Taiwan;
Scarlet fever

Abstract *Background:* A new sublineage of *emm*1 group A *Streptococcus* (GAS), $M1_{UK}$, has emerged in Europe, North America, and Australia. Notably, a significant portion of *emm*1 isolates in Asia, particularly in Hong Kong and mainland China, acquired scarlet fever-associated prophages following the 2011 Hong Kong scarlet fever outbreak. However, the presence of the $M1_{UK}$ sublineage has not yet been detected in Asia.
Methods: This study included 181 GAS isolates (2011—2021). The *emm* type of these isolates were determined, and 21 *emm*1 isolates from blood or pleural fluid (2011—2021) and 10 *emm*1 isolates from throat swabs (2016—2018) underwent analysis. The presence of the scarlet fever-associated prophages and the specific single nucleotide polymorphisms of the $M1_{UK}$ clone were determined by polymerase chain reaction and the genome sequencing.

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https://doi.org/10.1016/j.jmii.2024.01.004

Results: The $M1_{UK}$ lineage strains from throat swab and blood samples were identified. One of the $M1_{UK}$ strain in Taiwan carried the scarlet fever-associated prophage and therefore acquired the *ssa*, *speC*, and *spd1* toxin repertoire. Nonetheless, the increase of $M1_{UK}$ was not observed until 2021, and there was a reduction in the diversity of *emm* types in 2020—2021, possibly due to the COVID-19 pandemic restriction policies in Taiwan.
Conclusions: Our results suggested that the $M1_{UK}$ lineage clone has introduced in Taiwan. In Taiwan, the COVID-19 restrictions were officially released in March 2023; therefore, it would be crucial to continuously monitor the $M1_{UK}$ expansion and its related diseases in the post COVID-19 era.

Introduction

Streptococcus pyogenes (group A *Streptococcus*) is a human pathogen that causes diseases including tonsilitis, scarlet fever, pyoderma, necrotizing fasciitis, and toxic shock syndrome. Scarlet fever is a streptococcal superantigen-mediated disease and typically affects children aged 5—15.[1] Scarlet fever was related to significant mortality and morbidity in the 19th century; however, the incidence of this disease declined dramatically during the 20th century.[2] Intriguingly, the unexpected surge in scarlet fever cases has been reported in 2011 in Hong Kong and mainland China, and in 2014—2016 in England. Further, the increase of scarlet fever cases was documented in different geographical regions in this decade, including Singapore, South Korea, USA, Germany, and Australia.[3—7]

In Hong Kong and mainland China, the macrolide-resistant *emm*12 strain with the presence of novel prophages carried superantigens (SSA and SpeC) and DNase (Spd1) was notable in 2011 scarlet fever outbreak.[8—10] The epidemiological surveillance in Shanghai (China) showed that the prevalence of *emm*1 isolates increased from 3.8 % in 2011 to 48.5 % in 2014, and the mobile genetic elements confer the expression of SSA, SpeC, and resistance to tetracycline, erythromycin, and clindamycin, were detected to the *emm*1 isolates.[11,12] Furthermore, the nucleotide sequence of this *ssa*-positive 46-kb prophage in the selected *emm*1 isolates from mainland China shared 94 %—100 % identity compared with that in the *emm*12 HKU360, suggesting that the horizontal transfer of the prophage DNA would confer a new superantigen repertoire in the *emm*1 clone in mainland China.[11,12] Although the acquisition of the scarlet fever-associated prophages and transposable elements encoding multidrug resistance genes was identified in the majority of scarlet fever isolates,[13] Luk et al.[14] suggested that there is still insufficient evidence to conclude that a particular strain or virulence factor was associated with increased incidence or severity of scarlet fever in Hong Kong.

Different from the scarlet fever isolates identified in East Asia, various *emm* types, including *emm*3, *emm*12, *emm*4, and *emm*1 isolates were identified in the scarlet fever outbreak in the UK, 2014.[13,15,16] Most of these scarlet fever isolates were susceptible to erythromycin and clindamycin,[15] and no evidence suggested that the increased number of scarlet fever cases was a strain-specific or a mobile genetic element specific phenomenon.[17] Nonetheless, in 2016, with the coincidence of the seasonal rise of scarlet fever cases, the unexpected elevation of invasive GAS infections was notified in England, and the virulent *emm*1 strain, designed as $M1_{UK}$, was identified.[18] The $M1_{UK}$ strain is distinguished from pandemic *emm*1 isolates ($M1_{gobal}$) by 27 specific single-nucleotide polymorphisms and the increased expression of superantigen SpeA.[18] Currently, $M1_{UK}$ was reported in the Europe, Australia, and northern America regions, including England, Denmark, Netherland, Belgium, Portugal, Canada, and USA.[18—24] Noticeably, 26 % $M1_{UK}$ lineage isolates in Australia acquired the scarlet fever-associated, *ssa*-positive prophages.[22]

Taiwan is geographically close to Hong Kong and a significant upward trend for the overall scarlet fever outpatient rate was observed between 2009 and 2017.[25] Our study showed that the *ssa*-positive *emm*12 isolate was identified in northern Taiwan in 2012, and the prevalence of *ssa*-positive isolates increased from 6.3 % in 2008—2010 to 58.3 % in 2017—2019.[26] Nonetheless, different from the macrolide-resistant isolates circulated in Hong Kong and mainland China, most of the analyzed *emm*1 isolates were susceptible to erythromycin and clindamycin. Also, 47.6 % of analyzed *emm*1 isolates did not possess the scarlet fever-associated prophages.[26]

New M1 lineage clones have been reported in different geographic regions, and they have been associated with an increase in cases of GAS infection. This study aims to investigate whether these new M1 lineage clones, including $M1_{UK}$ and *emm*1 isolates acquiring the *ssa*-positive prophage, have emerged in Taiwan. Additionally, we compared the phylogenetic relationships of *emm*1 isolates in Taiwan, the UK, Hong Kong, and mainland China to reveal the origins of these M1 lineage clones.

Methods

Bacterial isolates and culture conditions

One-hundred and eighty-one GAS isolates were included in this study. Twenty-one *emm*1-type group A *Streptococcus* (GAS) isolates from blood or pleural fluid collected during 2011—2021 at the Chang Gung Memorial Hospital at Linkou (Taoyuan, Taiwan) and 10 *emm*1-type isolates from throat swabs collected during 2016—2018 at the National Cheng Kung University Hospital (Tainan, Taiwan) were further

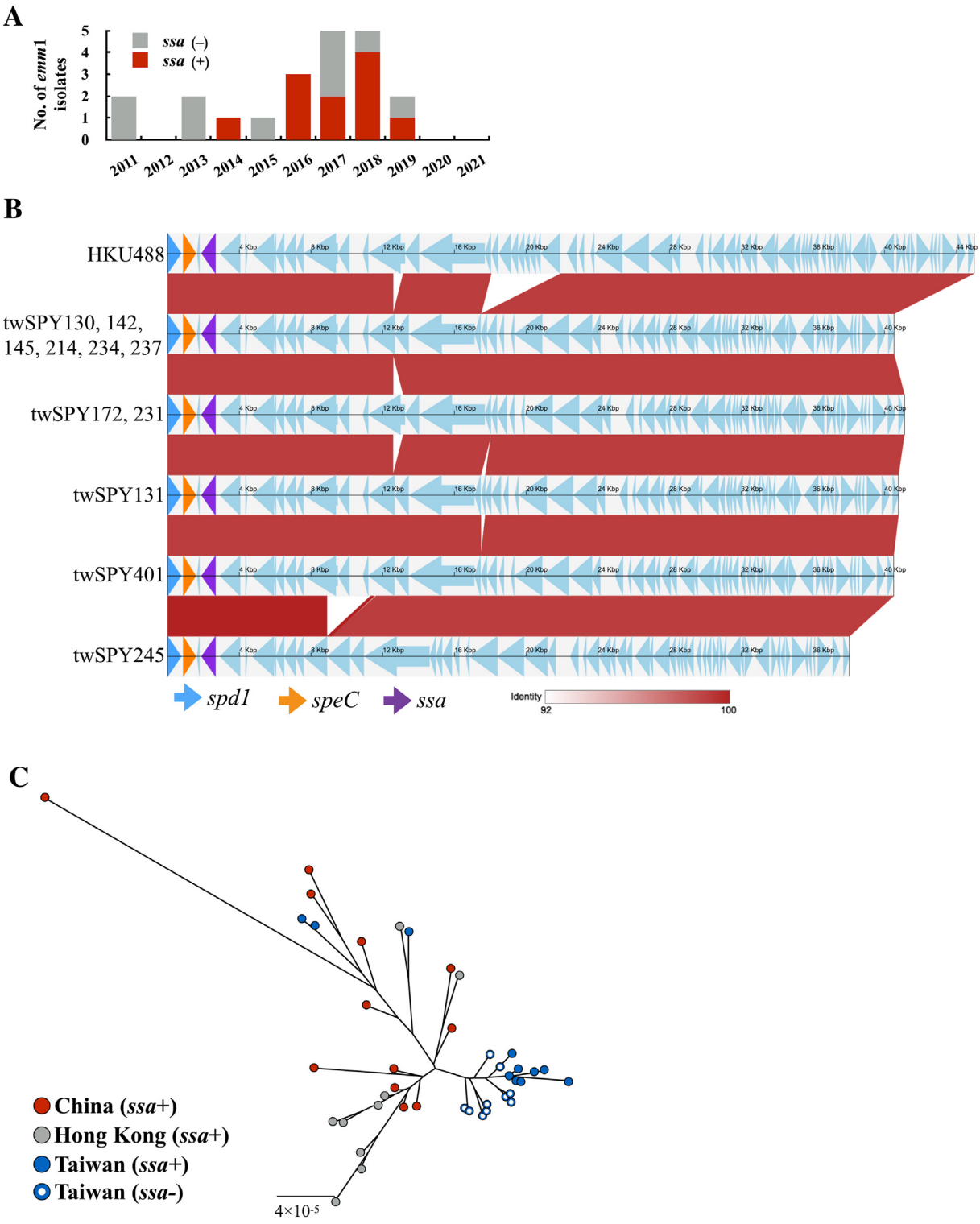

Figure 1. The similarity of *ssa*-positive 46-kb prophages and phylogenetic distance of the *emm*1 isolates in Taiwan, Hong Kong, and mainland China. (A) The prevalence of *ssa*-positive and *ssa*-negative *emm*1 isolates in Taiwan from 2011 to 2021. **(B)** Genetic organization of the *ssa*-positive 46-kb prophage from HKU488 (*emm*1) and *emm*1 isolates in Taiwan. The *spd1*, *speC*, and *ssa* are labeled as blue, orange, and purple arrows, respectively. Nucleotide sequence identity is graded from 100 % (red) to 92 % (white). **(C)** The maximum likelihood phylogenetic tree constructed from core single nucleotide polymorphisms (excluding prophage regions) of *emm*1 isolates in Taiwan (n = 21), Hong Kong (n = 9), and mainland China (n = 12). The scale bar indicates the nucleotide substitutions per site.

Table 1 The Taiwan *emm*1-type isolates used in this study.

Strain[a]	Year	*ssa*	Source	Lineage[b]
twSPY336	2011	−	Blood	$M1_{global}$
twSPY339	2011	−	Blood	$M1_{global}$
twSPY160	2013	−	Blood	$M1_{global}$
twSPY167	2013	−	Blood	$M1_{global}$
twSPY172	2014	+	Blood	$M1_{global}$
twSPY113	2015	−	Blood	$M1_{inter}$
twSPY130	2016	+	Blood	$M1_{global}$
twSPY131	2016	+	Blood	$M1_{global}$
twSPY214	2016	+	Blood	$M1_{global}$
twSPY142	2017	+	Blood	$M1_{global}$
twSPY145	2017	+	Blood	$M1_{global}$
twSPY143	2017	−	Blood	$M1_{global}$
twSPY144	2017	−	Blood	$M1_{global}$
twSPY149	2017	−	Blood	$M1_{inter}$
twSPY231	2018	+	Blood	$M1_{global}$
twSPY234	2018	+	Blood	$M1_{global}$
twSPY237	2018	+	Blood	$M1_{global}$
twSPY245	2018	+	Blood	$M1_{global}$
twSPY227	2018	−	Pleural fluid	$M1_{global}$
twSPY401	2019	+	Blood	$M1_{global}$
twSPY376	2019	−	Blood	$M1_{UK}$
S1-2823	2016	−	Throat	$M1_{global}$
S1-2861	2016	−	Throat	$M1_{global}$
S1-2879	2016	+	Throat	$M1_{global}$
S1-2905	2016	+	Throat	$M1_{global}$
S1-2906	2016	+	Throat	$M1_{global}$
S1-3045	2017	+	Throat	$M1_{inter}$
GAS-4	2018	+	Throat	$M1_{global}$
GAS-7	2018	+	Throat	$M1_{global}$
GAS-10	2018	+	Throat	$M1_{global}$
GAS-29	2018	+	Throat	$M1_{UK}$

[a] twSPY413: Spontaneous mutation in the *covS* gene.
[b] Among the throat isolates, the $M1_{inter}$ (S1-3045) and $M1_{UK}$ (GAS-29) clones were determined by whole genome sequencing, and the $M1_{global}$ lineage was determined by sequencing the *rofA* gene.

investigated. The *emm*1-type, $M1_{global}$ lineage A20 strain and GAS isolates from Chang Gung Memorial Hospital at Linkou were described elsewhere.[27] GAS isolates were cultured on trypticase soy agar containing 5 % sheep blood or in tryptic soy broth (Becton, Dickinson and Co., Sparks, MD, USA) supplemented with 0.5 % yeast extract (TSBY). The genome sequence of GAS isolates from Hong Kong, mainland China, and the UK was obtained from the European Nucleotide Archive Database (https://www.ebi.ac.uk/ena/browser/home), and the information regarding these isolates is listed in Supplementary Table S1.

DNA manipulations and *emm* typing

Genomic DNA used for polymerase chain reaction (PCR) and Illumina sequencing was extracted by the previously described methods.[28] The *emm* typing, including the PCR amplification, DNA sequencing, and assigning *emm* type to the isolates, were performed according to the protocol from the Centers for Disease Control and Prevention, USA (https://www.cdc.gov/streplab/groupa-strep/emm-background.html).

RNA manipulations and reverse transcription quantitative polymerase chain reaction (RT-qPCR)

RNA extraction and reverse transcription were performed as previously described.[28] Quantitative PCR (qPCR) was performed in a 20 μL reaction mixture containing 1 μL of cDNA, 0.8 μL of primers (10 μM), and 10 μL of SensiFAST SYBR Lo-ROX pre-mixture (Bioline Ltd; London, United Kingdom) according to the manufacturer's instructions. Biological replicate experiments were performed using two-independent RNA preparations in duplicates. The expression level of *speA* was detected by primers speA(qPCR)-F (5′- CTGTTACTCACGA-GAATGTGAAA -3′) and speA(qPCR)-R (5′- ATCA-TAATTTGGCCCTGAAA -3′), normalized to *gyrA*,[29] and analyzed using the $\Delta\Delta CT$ method (QuantStudio 3 System; Thermo Fisher Scientific Inc. Waltham, MA, USA). All values of the control (A20) and experimental groups (the clinical isolates) were divided by the mean of the control samples before statistical analysis.[30]

Genome sequencing and genomic analysis

Sequencing library was generated using TruSeq Nano DNA HT Sample Prep Kit (Illumina, San Diego, CA, USA) following manufacturer's recommendations. The DNA libraries were sequenced on Illumina NovaSeq 6000 platform and 150 bp paired end reads were generated by Genomics BioSci & Tech Co. (Taiwan). The raw reads were trimmed to clean reads with Trimmomatic v0.39. Bowtie 2 (Ver 2.5.1)[31] and Samtools (Ver 1.16.1) (http://www.htslib.org) distributed by Bioconda (Ver 23.1.0) (https://bioconda.github.io)[32] were used to map reads to the *emm*1 reference strain MGAS5005 (NCBI CP000017.2) or the 46-kb prophage in HKU360 (NCBI CP009612.1). The consensus sequence from assemblies was extracted by Unipro UGENE (Ver 46.0) (http://ugene.net)[33] and annotated by using Prokka.[34] The pan-genome of the *emm*1 isolates used in this study was queried using Roary (https://sanger-pathogens.github.io/Roary/).[35] The maximum-likelihood tree was generated by raxmlGUI (Ver 2.0.10) (https://antonellilab.github.io/raxmlGUI/)[36] based on the concatenated single nucleotide polymorphisms in the core genome and presented using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The nucleotide sequence similarity of the target region was compared by using Blastn (https://blast.ncbi.nlm.nih.gov/). The comparison diagrams of the sequences, based on the aligned segments and their identities from the hit table, were generated by Health GeneTech Corp (Taiwan) with the utilization of GenomeDiagram module of Biopython.[37]

Statistical analysis

Statistical analyses for qPCR experiments were performed using Prism software version 5 (GraphPad Software, Inc; San Diego, CA, USA). Significant differences between the

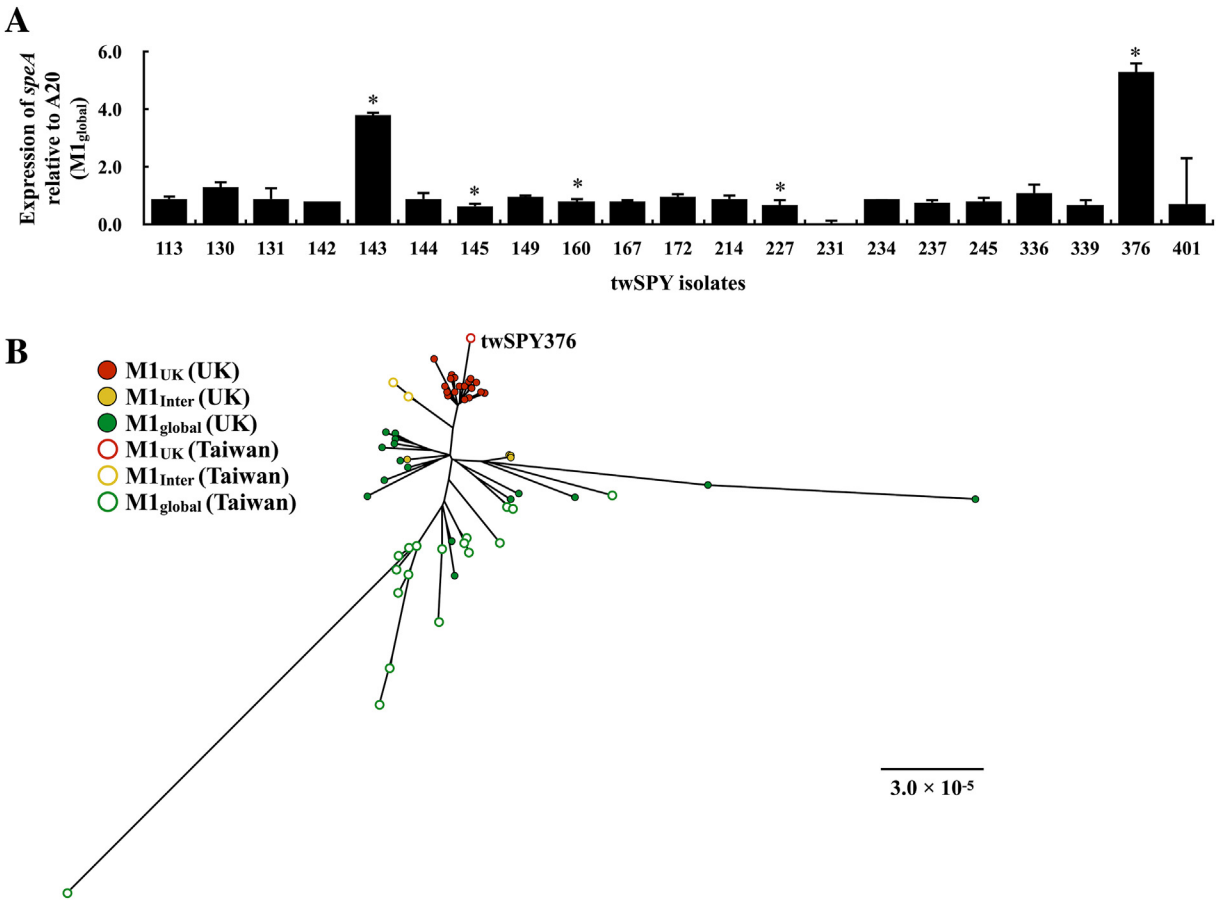

Figure 2. Identification of the $M1_{UK}$ lineage clone in the *emm*1 isolates in Taiwan. (A) The transcription level of *speA* in the *emm*1 isolates in Taiwan. GAS isolates were grown to $O.D._{600}$ = 1.0 and bacterial RNA was extracted for reverse transcription quantitative PCR analysis. The expression of *speA* was normalized to that of *gyrA*. twSPY231 is the *speA*-negative isolate. *, $P < 0.05$. **(B)** The maximum likelihood phylogenetic tree constructed from core single-nucleotide polymorphisms (excluding prophage regions) of *emm*1 isolates in Taiwan (n = 21) and $M1_{UK}$ (n = 19), $M1^{global}$ (n = 16), and $M1_{inter}$ (n = 4) in the UK. The scale bar indicates the nucleotide substitutions per site.

control and experimental groups were determined using Student's *t*-test. Statistical significance was set at $P < 0.05$.

Results

The scarlet fever-associated prophages and phylogenetic relationship of the *emm*1 GAS isolates

The sequence of the *ssa*-positive 46-kb prophage in our *emm*12 isolate (twSPY128) was identical to ΦHKU360 (Hong Kong) (Supplementary Fig. S1), suggesting that the Hong Kong scarlet fever lineage *emm*12 clone, or this scarlet fever-associated prophage, emerged in Taiwan. The prevalence of *ssa*-positive *emm*1 isolates was also increased in Taiwan[26]; in this study, the sequence of the 46-kb *ssa*-positive prophage in *emm*1 isolates were analyzed. During 2011–2021, a total of 21 invasive *emm*1-type isolates were identified; among them, eleven isolates were *ssa*-positive (Fig. 1A). To investigate the origin of these *ssa*-positive *emm*1 isolates, the sequence similarity of the *ssa*-positive prophages between our *emm*1 isolates and the *emm*1 isolate HKU488 was compared. The *spd1*, *speC*, and *ssa* genes were identified in all sequenced *emm*1 isolates (Fig. 1B). Different from HKU488 in Hong Kong, the sequence of the 46 kb prophages in our *emm*1 isolates was not identical to that of HKU360, and the sequence identity was ranged from 84 % to 91 % (Fig. 1B). We next performed phylogenetic analyses based on the core genome sequence to evaluate the clonal relationships among Taiwan (21 isolates; Table 1), Hong Kong (9 isolates), and mainland China (12 isolates) *ssa*-positive *emm*1 isolates (Supplementary Table S1). The maximum likelihood phylogenetic analysis showed that three *ssa*-positive Taiwan *emm*1 isolates (twSPY142, twSPY145, and twSPY172) were phylogenetically closely related to Hong Kong and mainland China *emm*1 isolates (Fig. 1C). Noticeable, eighteen of 21 Taiwan *emm1* isolates were phylogenetically separated from Hong Kong and mainland China *emm*1 isolates, and the *ssa*-positive and *ssa*-negative Taiwan *emm*1 isolates were distributed in the same cluster (Fig. 1C). These results suggested that the horizontal transfer of the *ssa*-positive scarlet fever-associated prophages could occur among local *emm*1 isolates in Taiwan.

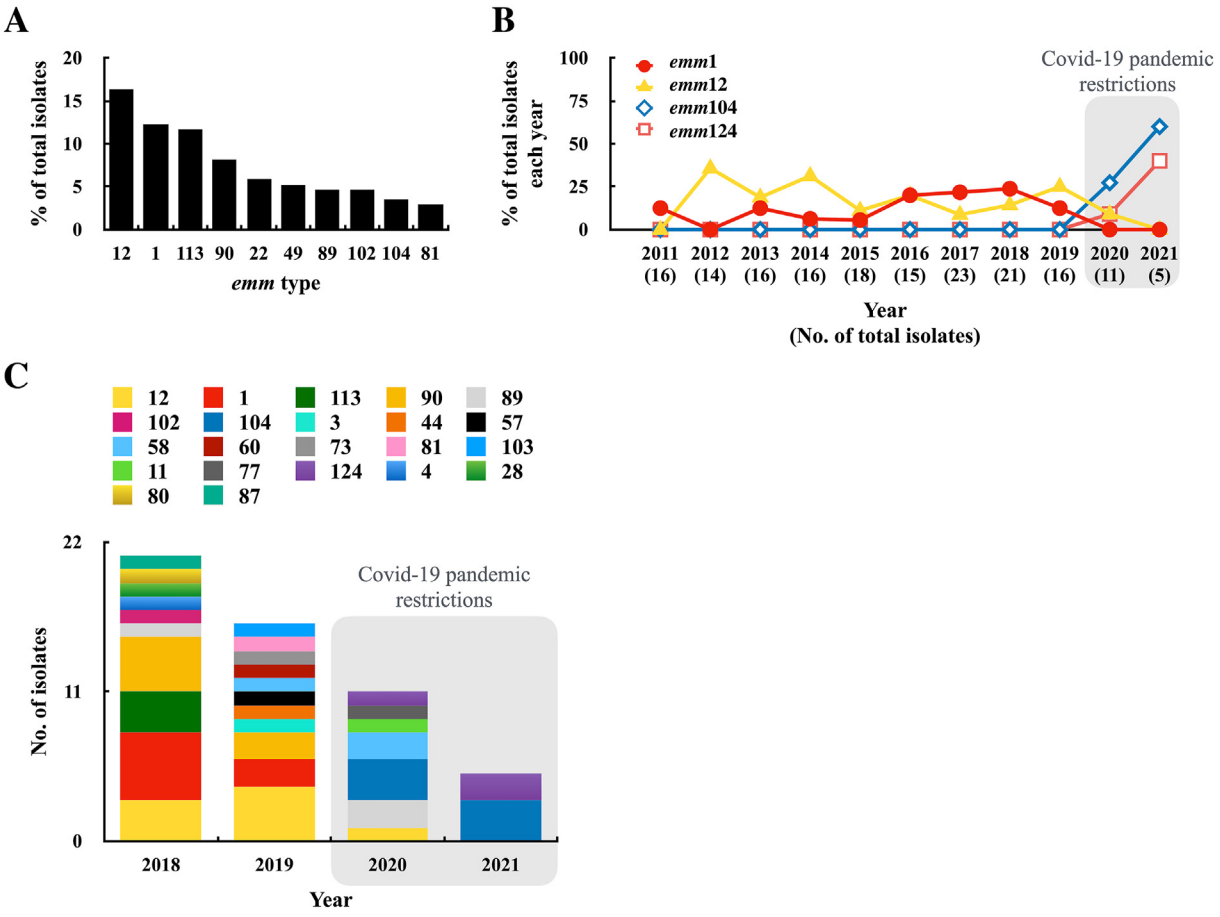

Figure 3. The prevalence of *emm*1, *emm*12, *emm*104, and *emm*124 during 2011—2021. (A) The ten most prevalent *emm* types during 2011—2021. **(B)** The decrease of *emm*1 and *emm*12 and the surge of *emm*104 and *emm*124 during the COVID-19 pandemic restrictions (2020—2021) in Taiwan. **(C)** The diversity of *emm* types before (2018—2019) and after (2020—2021) COVID-19 pandemic restrictions.

Identification of $M1_{UK}$ lineage GAS in Taiwan

To investigate whether the *emm*1 isolates in Taiwan could belong to the $M1_{UK}$ lineage, the expression of *speA* of 21 *emm*1 isolates were analyzed by the RT-PCR. To compare to the $M1_{global}$ A20 strain, twSPY145, twSPY160, twSPY227, and twSPY231 (the *speA*-negative isolate) showed the lower *speA* expression level; however, two isolates (twSPY143 and twSPY376) had a 3.8—5.3-fold increased *speA* expression (Fig. 2A). TwSPY143 has the spontaneous inactivating mutation in the *covS* gene and therefore had the increase of *speA* expression.[27] Whole genome sequencing analysis showed that twSPY376 had 27 unique single nucleotide polymorphisms (SNPs) identical to the $M1_{UK}$ clone. Among 21 Taiwan *emm*1 isolates, 18 isolates were $M1_{global}$ lineage. Two isolates (twSPY113 and twSPY149) had 13 of 27 specific SNPs in $M1_{UK}$ lineage and were designated to $M1_{inter}$ (intermediate; Table 1) as indicated by the previous study.[18] Furthermore, we performed phylogenetic analyses based on the core genome sequence to evaluate clonal relationships among Taiwan *emm*1 (21 isolates) and the $M1_{global}$ (16 isolates), $M1_{inter}$ (4 isolates), and $M1_{UK}$ (19 isolates) lineage isolates from the UK (Table S1). TwSPY376 was clustered with $M1_{UK}$ lineage isolates and phylogenetically separated from $M1_{global}$ lineage isolates (Fig. 2B), further indicating that twSPY376 was the $M1_{UK}$ lineage clone.

Changes in the *emm* type prevalence during COVID-19 pandemic restrictions

In England, the $M1_{UK}$ clone represented 91 % of invasive *emm*1 isolates in 2020.[38] The $M1_{UK}$ lineage *emm*1 isolate was identified in Taiwan in 2019; however, there was no *emm*1 isolate can be found in 2020 and 2021 (Fig. 1A). From 2011 to 2021, the most prevalent *emm* types are *emm*12 (n = 28, 16 %) and *emm*1 (n = 21, 12 %) (Fig. 3A); therefore, it was unusual that only one *emm*12 and no *emm*1 isolate was identified during 2020—2021. The outbreak of COVID-19 started in late 2019. In 2018, *emm*1 (n = 5, 24 %), *emm*12 (n = 3, 14 %), *emm*90 (n = 4, 19 %), and *emm*113 (n = 3, 14 %) were the prevalent types, and *emm*1 (n = 2, 12.5 %), *emm*12 (n = 4, 25 %), and *emm*90 (n = 2, 12.5 %) were the prevalent types in 2019 (Fig. 3B and C). There were 10 and 11 different *emm*-types were identified in 2018 and 2019, respectively (Fig. 3C). Nonetheless, 7 and 2 different *emm*-types were identified in 2020 and 2021, respectively. In 2020, the most prevalent *emm*-type was *emm*104 (n = 3, 27.3 %). The *emm*104 and *emm*124, which

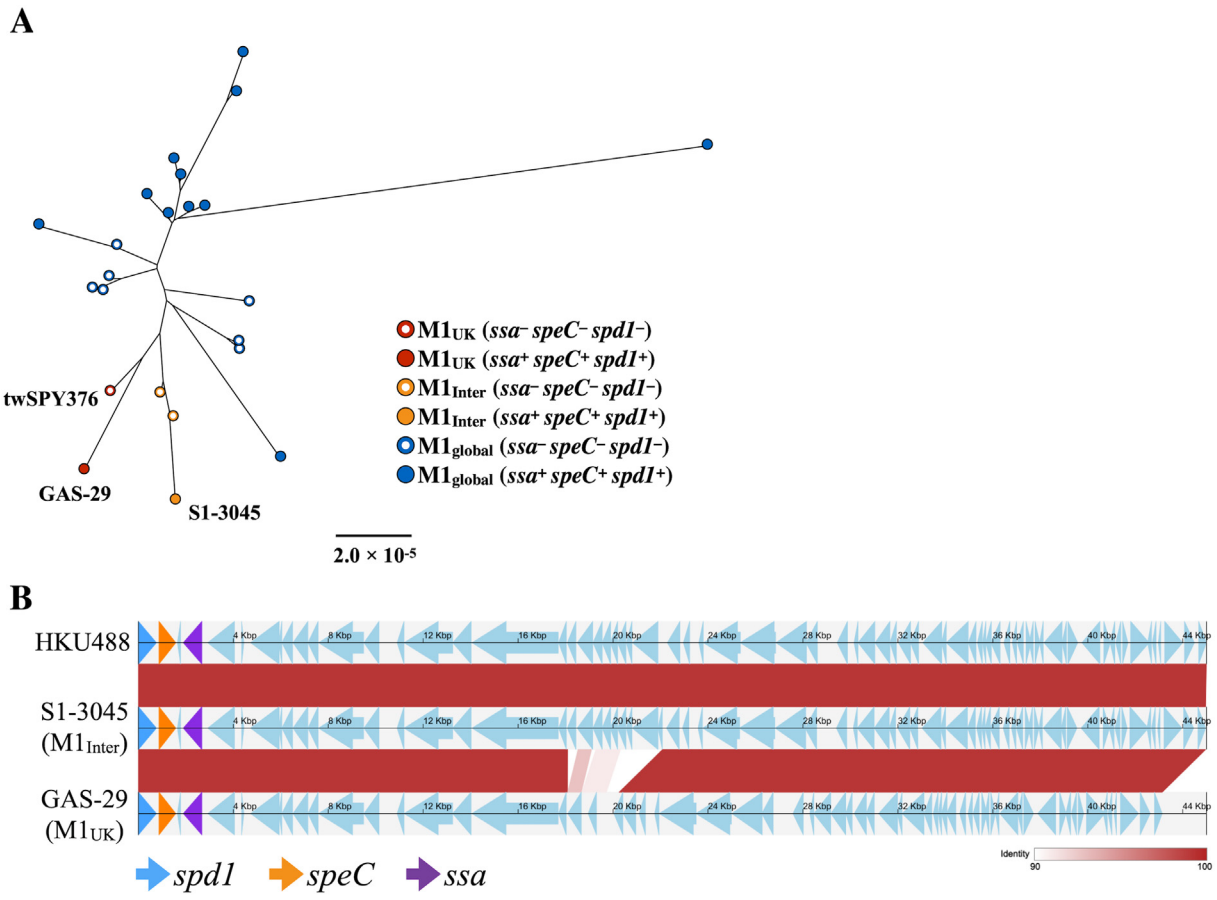

Figure 4. The phylogenetic distance of the *emm*1 isolates in Taiwan and the similarity of *ssa*-positive 46-kb prophages in $M1_{UK}$, $M1_{Inter}$, and ΦHKU488. (A) The maximum likelihood phylogenetic tree constructed from core single-nucleotide polymorphisms (excluding prophage regions) of Taiwan M1 isolates (n = 23). The scale bar indicates the nucleotide substitutions per site. **(B)** Genetic organization of the *ssa*-positive 46-kb prophage from HKU488 (*emm*1), $M1_{UK}$, and $M1_{inter}$ lineage isolates in Taiwan. The *spd1*, *speC*, and *ssa* are labeled as blue, orange, and purple arrows, respectively. Nucleotide sequence identity is graded from 100 % (red) to 90 % (white).

were not identified during 2011–2019, were the *emm*-types that can be found in 2021 (Fig. 3C). These results indicate that the number of invasive GAS isolates and the diversity of *emm* types were decreased during the COVID-19 restrictions.

The $M1_{UK}$ lineage clone in Taiwan carried the scarlet fever-associated prophage

No *emm*1-type isolate was found among invasive *emm*1 isolates during 2020–2021. To verify whether the $M1_{UK}$ lineage clone has introduced in Taiwan, ten pharyngeal *emm*1 isolates identified during 2016–2018, were included in this study (Table 1). The *ssa* gene can be detected by PCR in 8 of these isolates (except S1-2823 and S1-2861, Table 1). Three $M1_{UK}$ specific SNPs in the *rofA* gene were found in two isolates (S1-3045 and GAS-29, Table 1). Whole genome sequencing results showed that GAS-29 was the $M1_{UK}$ lineage strain and S1-3045 was the $M1_{inter}$ clone (Fig. 4A). Also, the upregulation of *speA* was only observed in GAS-29 (4.7 ± 0.84-fold increase compared to the $M1_{global}$ A20 strain) but not S1-3045. Both these two isolates carried the scarlet fever-associated prophages with 95–99 % sequence identity compared to ΦHKU488 (Fig. 4B).

Discussion

$M1_{UK}$ is a newly emerged lineage of *emm*1 GAS and is epidemiologically related to invasive infections in the UK.[18,39] In Asia, especially in Hong Kong and mainland China, the predominant *emm*1 strains were $M1_{global}$ strains carried the scarlet fever-associated prophages encoding SSA, SpeC, and Spd1,[11,40,41] and the $M1_{UK}$ lineage clone has not been reported yet. This study identified the $M1_{UK}$ lineage strains in Taiwan. Furthermore, similar to the report in Australia,[22] the $M1_{UK}$ clone in Taiwan acquired the scarlet fever-associated prophage. These results suggest that the $M1_{UK}$ strains have introduced to the Pacific Asia region.

After being identified, the $M1_{UK}$ lineage clone expanded rapidly and replaced the $M1_{global}$ lineage as the prevalent M1 clone in the UK and Australia.[18,22,38] We identified $M1_{UK}$ lineage clone among GAS isolates in 2018–2019 in Taiwan.[26] Intriguingly, during 2020–2021, no *emm*1 isolates were

found in our collections. COVID-19 restrictions were introduced in the late 2019 in Taiwan. Masking and social distancing not only prevents people from COVID-19 infection but also could reduce GAS transmission among population. Nonetheless, the upsurge trend of $M1_{UK}$ prevalence was observed in the UK and Netherlands after releasing the COVID-19 restrictions.[38,42] In Taiwan, the COVID-19 restrictions were officially released in the early of 2023. Our results indicated that the $M1_{UK}$ lineage clone has been detected in Taiwan; therefore, it would be crucial to continuously monitor the $M1_{UK}$ expansion and its related diseases in the post COVID-19 era.

The scarlet fever-associated prophages, such as ΦHKU.vir and ΦHKU.488, encode the secreted superantigens SSA and SpeC and the DNase Spd1.[10,12] After 2011 Hong Kong scarlet fever outbreak, the prevalence of the SSA-positive GAS, especially in *emm*12 and *emm*1 isolates, increased dramatically in Asia. Our previous study showed that the prevalence of SSA-positive isolates in Taiwan increased from 6.5 % in 2011—2013 to 40 % in 2017—2019.[26] In mainland China, Yu et al.[40] showed that 99 % (104/105) *emm*1 and 98.5 % (198/201) *emm*12 isolates during 2016—2018 in Shenzhen carried the *ssa* gene. In Beijing, 85 % *emm*1 (85/100) and 77.6 % *emm*12 (111/143) isolates in 2019 are SSA-positive.[41] Davies et al.[13] suggest that the acquisition of *ssa* could be related to the expansion of scarlet fever-associated *emm*12 lineages in Hong Kong. Nonetheless, Brouwer et al.[43] showed that SpeC and Spd1, but not SSA, act synergistically to facilitate GAS nasopharyngeal colonization in a mouse model. Luk et al.[14] suggested that there is still no solid evidence to support the association between the increased scarlet fever incidence and the presence of specific foreign genetic elements. Although the expansion of *ssa*-positive GAS indicates that the acquisition of the scarlet fever-associated prophages would increase the evolution fitness of GAS, the mechanisms of these prophages participate in GAS pathogenesis need to be investigated.

In Australia, 26 % of $M1_{UK}$ strains have acquired the scarlet fever-associated prophages.[22] Based on the fact that these prophages expand quickly among $M1_{global}$ isolates, it would be expected that the prevalence of SSA-positive $M1_{UK}$ isolates may increase gradually in Taiwan. Emerging and expansion of $M1_{UK}$ strains in the UK is epidemiologically linked to the increase in invasive GAS infections.[18,39] The present study identified the $M1_{UK}$ strain carried the ΦHKU.vir-like prophage in Taiwan; however, the impact of the emerging $M1_{UK}$ and ΦHKU.vir-like prophages-positive $M1_{UK}$ strains to the society in Taiwan had not revealed yet. Furthermore, since the $M1_{UK}$ strains have been identified in Europe, North America, Australia,[23,24,38] and Asia (Taiwan), the emerging of this lineage strains would be considered as the global event. Importantly, with the release of COVID-19 restrictions worldwide, the potential threat from the $M1_{UK}$ lineage clones to the society would be a significant concern shortly.

Declaration of conflict of interest

The authors affirm no conflict of interest relative to any source of funding, sponsorship, or financial benefit.

Acknowledgements

We are grateful to the Bacterial Bank (Chang Gung Memorial Hospital at Linkou, Taiwan) and National Cheng Kung University Hospital (Tainan, Taiwan) for providing clinical isolates. This work was supported by parts of grants from the Chang Gung Memorial Hospital at Linkou, Taiwan (CORPD1M0011) and National Science and Technology Council, Taiwan (112-2320-B-182-002 and 112-2628-B-182-004).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.01.004.