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Short Communication

High-level levofloxacin-resistant *emm*12 group A *Streptococcus*, 2012–2018: A multicenter study in Taiwan

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Abstract High-level levofloxacin-resistant group A *Streptococcus* emerged in Taiwan in 2012. Among the 24 isolates identified, 23 belonged to *emm*12/ST36, most harbored the same GyrA and ParC mutations and were highly clonal. wgMLST showed them to be closely related to the Hong Kong scarlet fever outbreak strains. Continuous surveillance is warranted.

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Introduction

Streptococcus pyogenes, commonly known as group A *Streptococcus* (GAS), colonizes human mucosal and skin epithelium, and causes mild to severe infections in children and adults.[1] GAS infections are a significant public health and economic burden globally. The M protein encoded by the *emm* gene is a major virulence determinant of GAS and 200 different *emm* types have been reported.[1,2]

There has been a resurgence of GAS infections in recent years.[1,2] In Hong Kong and mainland China, a scarlet fever outbreak occurred in 2011, and remained at an elevated level in 2015, with multidrug-resistant *emm*12 strains being the predominant lineage responsible for the outbreak.[3] A recent single center study also reported *emm*12 to account for 79.2% of GAS from pediatric patients with upper respiratory tract infections during 2010—2019 in southern Taiwan.[4]

The most common antibiotics used to treat GAS infections are β-lactams, with macrolides and lincosamides being alternatives. However, high rates of macrolide and/or lincosamide resistance have been reported in different parts of the world including Taiwan.[1,2,4] For patients with β-lactams allergy, a more suitable antimicrobial is fluoroquinolones to treat severe infections, but fluoroquinolone-nonsusceptible GAS isolates have been detected in different countries. However, most of those isolates displayed low-level fluoroquinolone resistance and high-level resistance in GAS remained uncommon.[2] The present study reports the transient rise and spread of a high-level levofloxacin-resistant *emm*12 GAS clone in Taiwan.

Methods

Isolate collection and identification

GAS isolates were collected as part of the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program from July to September biennially between 2012 and 2018 from 27 hospitals located in different regions of Taiwan. Isolate collection and identification followed previously described protocols.[5]

Antimicrobial susceptibility testing and *emm* typing

Minimum inhibitory concentrations (MICs) of various antimicrobial agents were determined by broth microdilution (Thermo Fisher Scientific, UK) following the guidelines of CLSI.[6] Isolates with levofloxacin MIC >2 μg/mL were considered nonsusceptible and were further tested by E-test (bioMérieux, France). All GAS isolates were characterized by the M protein serotypes using the US CDC *emm* typing protocols (https://www.cdc.gov/streplab/groupa-strep/resources.html#typing-protocol).

Characterization of levofloxacin-nonsusceptible isolates

All fluoroquinolone-nonsusceptible GAS isolates were checked for mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* using published primers.[7] Pulsed-field gel electrophoresis (PFGE) was performed using restriction endonuclease SgrAI as described previously and analyzed to investigate the clonal relationships.[5] Multilocus sequence typing (MLST) was performed following standard MLST protocol (https://pubmlst.org/organisms/streptococcus-pyogenes).

Whole genome MLST (wgMLST)

To investigate the evolutionary relationships of *emm*12 GAS in East Asia, we first performed whole genome sequencing on three *emm*12 GAS isolates from this study. Genome sequencing was performed using an Illumina NovaSeq with 150-bp paired-end reads. Whole-genome allelic profiles of our three isolates and 28 *emm*12 isolates, whose sequences were downloaded from public databases and consisted of isolates mostly from Hong Kong,[3] were determined and constructed for phylogenetic analysis in BioNumerics 8.1 (Applied Math, Belgium). After genome assembly, acquired antimicrobial resistance genes and exotoxin genes were detected using ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/) and manual BLASTN analysis, respectively. Genome sequences of the three GAS isolates have been deposited at the NCBI with accession numbers: JANCAA000000000 (S06-283); JANCAB000000000 (N21-264); JANCAC000000000 (S01-268).

Results

Isolates, antimicrobial susceptibility, and *emm*12 prevalence

A total of 593 non-duplicate GAS isolates were collected during 2012—2018. Around one third of the isolates were from <18 y. o. (n = 224, 37.8%). The most common specimen types were abscess/pus/wound (45.7%), followed by respiratory tract (30.2%) and blood (20.9%). The 593 isolates belonged to 42 different *emm* types (data not shown). *emm*12 was the most frequently occurring type overall (24.8%) and its proportion increased gradually over the study years, from 18.4% in 2012 to 27.7% in 2018 (Table 1).

Penicillin, ceftriaxone, cefepime, meropenem, vancomycin, and daptomycin remained active against all tested isolates. The overall susceptibility to clindamycin, erythromycin, tetracycline was 79.4, 76.4, and 33.7%, respectively (Table 1). However, compared to early period (2012—2014), isolates from 2016 to 2018 were significantly less susceptible to clindamycin and erythromycin, but were more susceptible to tetracycline (all $p < 0.001$) (Table 1). Levofloxacin susceptibility was 96% overall but was significantly lower in 2012—2014 (92.8%) than in 2016—2018 (99.3%, $p = 0.001$).

Clinical characteristics and molecular characterization of levofloxacin-nonsusceptible GAS isolates

Among the 24 GAS isolates with levofloxacin nonsusceptibility, the majority (n = 21, 87.5%) had high-level levofloxacin resistance with MIC ≥24 μg/mL (Fig. 1a). Most (17, 70.8%) of these isolates were from respiratory tract specimens (14

Table 1 Source and antimicrobial susceptibility of 593 Group A *Streptococcus* isolates from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program.

Characteristics	No. of isolates (%)					*p* value[a] (2012–2014 vs. 2016–2018)
	2012–2018	2012	2014	2016	2018	
Total	593 (100.0)	136 (11.2)	171 (14.1)	145 (12.0)	141 (11.6)	
Age group[b]						0.002
<18 y.o	224 (37.8)	43 (31.6)	62 (36.3)	58 (40.0)	61 (43.3)	
18-64 y.o	250 (42.2)	51 (37.5)	71 (41.5)	67 (46.2)	61 (43.3)	
≥65 y.o	118 (19.9)	42 (30.9)	37 (21.6)	20 (13.8)	19 (13.5)	
Unknown	1 (0.2)	0	1 (0.6)	0	0	
Specimen types[c]						0.109
Abscess/pus/wound	271 (45.7)	65 (47.8)	85 (49.7)	63 (43.4)	58 (41.1)	
Blood	124 (20.9)	39 (28.7)	30 (17.5)	32 (22.1)	23 (16.3)	
Respiratory tract	179 (30.2)	28 (20.6)	52 (30.4)	46 (31.7)	53 (37.6)	
Others	19 (3.2)	4 (2.9)	4 (2.3)	4 (2.8)	7 (5.0)	
Susceptibility rate						
Clindamycin[d]	471 (79.4)	121 (89)	140 (81.9)	97 (66.9)	113 (80.1)	<0.001
Erythromycin	453 (76.4)	115 (84.6)	138 (80.7)	92 (63.4)	108 (76.6)	<0.001
Tetracycline	569 (33.7)	20 (14.7)	65 (38.0)	55 (37.9)	60 (42.6)	<0.001
Levofloxacin	569 (96.0)	124 (91.2)	161 (94.2)	144 (99.3)	140 (99.3)	0.001
*emm*12 prevalence	147 (24.8)	25 (18.4)	44 (25.7)	39 (26.9)	39 (27.7)	0.176

[a] *p* value was calculated by chi-square test comparing GAS isolates from early (2012–2014) vs later (2014–2018) period.
[b] The patient age of one isolate each in 2012 and 2014 was unknown, but the one in 2012 was from pediatric based on the record provided by the hospital.
[c] The 170 respiratory specimens included 157 throat/nasal swab, 13 sputum, and 4 unspecified. Others included 10 isolates from urine, 8 from aseptic body fluid, 1 from cervical discharge.
[d] Isolates that were clindamycin-susceptible and erythromycin-resistant were tested for inducible clindamycin resistance, and those tested positive were considered clindamycin-nonsusceptible.

throat and 3 sputum). All but one (23, 95.8%) of these 24 isolates belonged to *emm*12. Multivariate logistic regression showed that isolates belonging to *emm*12 type (OR 283.36, CI 25.43–3157.20, $p < 0.001$) were independent factor associated with levofloxacin nonsusceptibility.

PFGE showed one main cluster encompassing 21 (87.5%) isolates sharing >90% similarity including 17 isolates with indistinguishable PFGE patterns, indicating high clonality (Fig. 1a). MLST conducted on 9 isolates selected from this cluster revealed them to all belong to ST36. The highly clonal *emm*12/ST36 cluster contained isolates from different hospitals in different regions of Taiwan. The remaining three isolates with heterogeneous PFGE patterns included one *emm*12/ST36, one *emm*12/ST1281 (a new single locus variant of ST36), and one *emm*89/ST646 isolate.

The most common QRDR mutation profile among these levofloxacin-nonsusceptible GAS isolates (N = 21, 87.5%) was the combination of GyrA (S81F) and ParC (S79F) with levofloxacin MIC >32 μg/mL (Fig. 1a). The remaining 3 isolates with levofloxacin MICs in the 4–6 μg/mL range had different QRDR mutation profiles. Two (8.3%) and 19 (79.2%) of the levofloxacin-nonsusceptible isolates were also nonsusceptible to erythromycin and tetracycline, respectively.

Genomic comparison of Taiwan *emm*12 strains with the Hong Kong outbreak *emm*12 strains

wgMLST phylogenetic analysis was performed to compare *emm*12 strains from Taiwan with those from other countries. The 3 strains from Taiwan included 2 levofloxacin-resistant strains, one from 2012 (S01-268) and the other from 2018 (S06-283) (see Fig. 1a), plus a levofloxacin-susceptible strain from 2012 (N21-264). The other 28 strains were from Hong Kong (n = 23), Australia (n = 2), mainland China (n = 2), and USA (n = 1). Among these 31 *emm*12 GAS isolates, four major genetic lineages (clades I–IV) were present. The three isolates from Taiwan were closest to those from Hong Kong, mainland China, and an isolate from Australia in clade I and clade II (Fig. 1b). Our isolate S01-268, a representative strain from the highly clonal PFGE cluster of isolates, shared 96.7% allelic similarity with the Hong Kong scarlet fever outbreak strains. All three Taiwan isolates also carried exotoxin genes including *ssa*, *speC*, *spd1*, *sda*, *speG*, *speH*, *speI*, *smeZ*, *speB*, except the *ssa* gene that is missing in S01-268 isolate.

Discussion

The present study indicated that *emm*12, found to be the most prevalent type of GAS in Taiwan by our analysis and reported by others,[4,8] was associated with high-level levofloxacin resistance. Most of the levofloxacin-resistant strains were *emm*12/ST36, carried the same QRDR mutations in GyrA and ParC, and are highly clonal based on PFGE, despite being from different study years, specimen types, age groups, and geographic regions of Taiwan.

Although levofloxacin-nonsusceptible GAS has been detected in different regions of the world, most were low-level or borderline resistant (levofloxacin MIC 2–8 μg/mL) with a single QRDR mutation in ParC, and belonged to

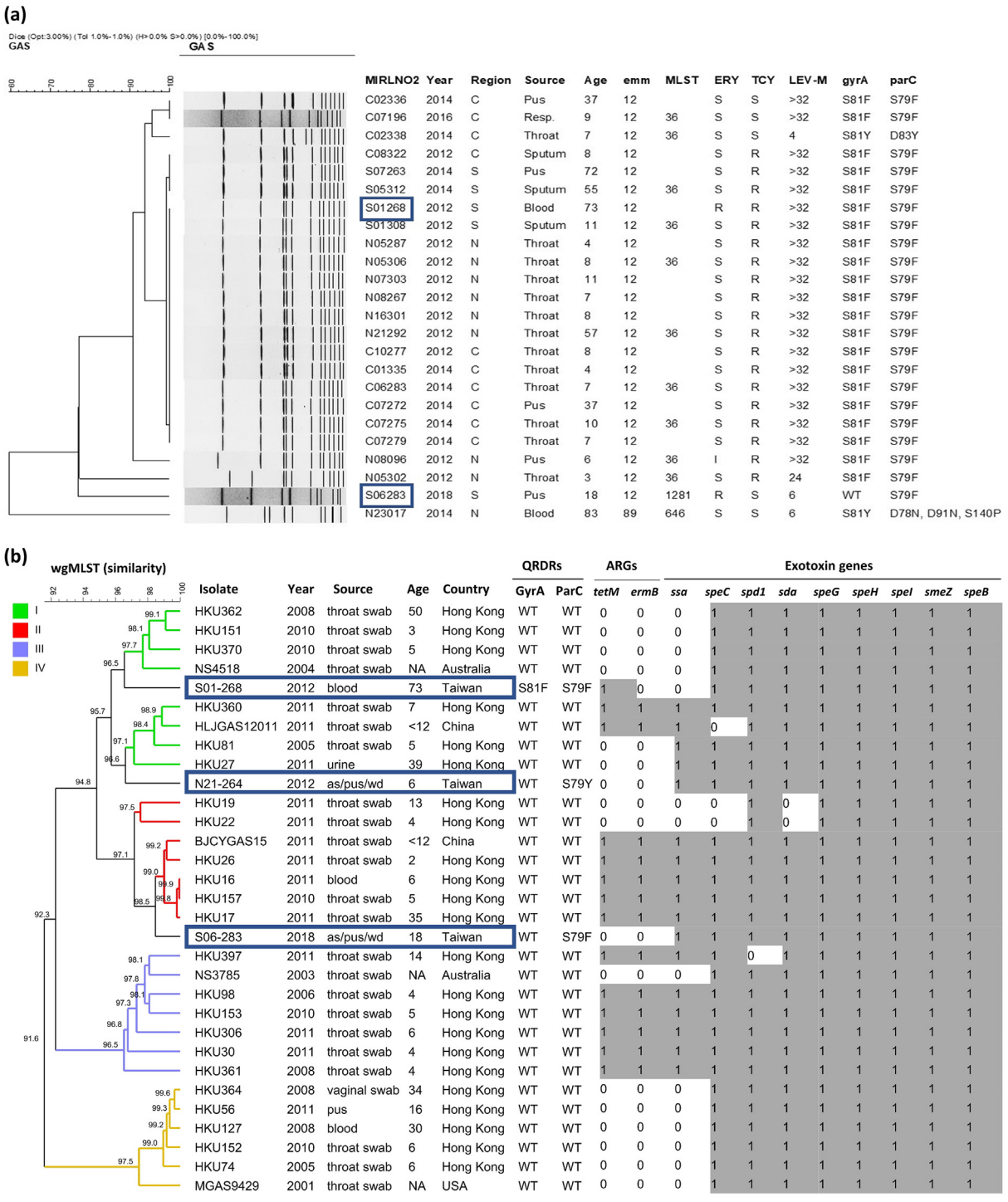

Fig. 1. (a) Dendrogram of 24 levofloxacin-resistant Group A *Streptococcus* isolates based on SgrAI-digested PFGE patterns. MIRLNO2, the first digit indicates the region of the hospital, the second and third digits indicated hospital code. LEV-M, levofloxacin MIC in μg/mL by Etest; Rectangle, isolates selected for whole genome sequencing. (b) Dendrogram of 31 *emm*12 Group A *Streptococcus* (GAS) strains using a whole-genome multilocus sequence type (wgMLST) scheme. Characterizations of 28 selected *emm*12 GAS isolates belonging to clade I through clade IV (colored legend in Figure) were obtained from a previous report for comparison.[3] Draft genomes or short read sequences of these 28 strains were retrieved according to their deposited numbers in public databases. Allelic information from wgMLST of our three (marked with blue rectangles) and the public 28 strains were clustered using the UPGMA method and a dendrogram was constructed. The scale of similarity is on the top of the dendrogram. QRDRs: quinolone resistance-determining regions; ARGs: antimicrobial resistance determinants; WT: wild-type; "0": gene absent; "1": gene present.

different *emm* types.[2] In a collection of GAS isolates between 2005 and 2012 from southern Taiwan, 33 levofloxacin-nonsusceptible *emm*12/ST36 GAS isolates were identified, but only 2 isolates had high-level fluoroquinolone resistance (levofloxacin MIC >32 μg/mL) and the rest had MIC 2 μg/mL.[8] Our finding of multiple GAS isolates with high-level levofloxacin MIC associated with GyrA and ParC double mutations, plus the increasing macrolide resistance shown in this study and recently reported by others[4] add to the concern of multidrug-resistant GAS. The medical personnel in Taiwan should be more alert in using non β-lactam antimicrobials in treating patients with GAS infection.

GAS superantigens SpeA, SpeC and SSA carried on virulence enhancing prophages have been associated with the scarlet fever outbreak *emm*12 strains in mainland China and Hong Kong. A recent study revealed how these exotoxins may provide fitness advantage to facilitate the re-emergence of GAS infections globally.[3] Our genome-wide comparison showed that the Taiwan *emm*12 strains also carried these exotoxin genes and belonged to the same clades as the Hong Kong and mainland China outbreak strains, which suggested that these *emm*12 strains shared a similar ancestor.

The reason for the rise and spread of the high-level levofloxacin-resistant *emm*12/ST36 clone during 2012—2014 in Taiwan and its decrease in 2016—2018 may be related to changes in fluoroquinolone use. It is well established that stepwise QRDR mutations can emerge rapidly in bacteria exposed to fluoroquinolones.[2,7] In Taiwan, previous studies have found possible underreporting of outpatient fluoroquinolone use.[9] Awareness of increasing fluoroquinolone resistance and subsequent advocacy for judicious fluoroquinolone use in recent years may have resulted in decreased non-public health insurance covered fluoroquinolone use.[9,10]

In conclusion, this nationwide longitudinal study identified a high-level levofloxacin-resistant *emm*12/ST36 GAS clone in Taiwan. The close relatedness of the *emm*12 strains in Taiwan to the scarlet fever epidemic strains in Hong Kong indicates the need for close monitoring of GAS epidemiology in our region.

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Ethical Approval

The isolates were collected from clinical samples as part of standard care and the Taiwan Surveillance of Antimicrobial Resistance (TSAR) project was approved by the Research Ethics Committee of National Health Research Institutes, Taiwan (EC1010602-E, EC1030406-E and EC1050606-E).

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Some results from this study were presented as a poster at the ICAAC 2015 in San Diego, CA, United States.

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