**Comparative Genomics of Community-Acquired ST59 Methicillin-Resistant Staphylococcus aureus in Taiwan: Novel Mobile Resistance Structures with IS1216V**

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**Abstract**

Methicillin-resistant *Staphylococcus aureus* (MRSA) with ST59/SCCmecV and Panton-Valentine leukocidin gene is a major community-acquired MRSA (CA-MRSA) lineage in Taiwan and has been multidrug-resistant since its initial isolation. In this study, we acquired the mechanism of multidrug resistance in an ST59 CA-MRSA strain (PM1) by comparative genomics. PM1’s non-β-lactam resistance was encoded by two unique genetic traits. One was a 21,832-bp composite mobile element structure (MESPM1), which was flanked by direct repeats of enteroococcal IS1216V and was inserted into the chromosomal *sasK* gene; the target sequence (att) was 8 bp long and was duplicated at both ends of MESPM1. MESPM1 consisted of two regions: the 5'-end side 12.4-kb region carrying Tn551 (with *ermB* and Tn5405-like (with *aph*3’')-IbLL and *aadE*), similar to an *Enterococcus faecalis* plasmid, and the 3'-end side 6,587-bp region (MEScat) that carries *cat* and is flanked by inverted repeats of IS1216V. MEScat possessed att duplication at both ends and additional two copies of IS1216V inside. MESPM1 represents the first enteroococcal IS1216V-mediated composite transposon emerged in MRSA. IS1216V-mediated deletion likely occurred in IS1216V-rich MESPM1, resulting in distinct resistance patterns in PM1-derivative strains. Another structure was a 6,025-bp *tet* carrying element (MEStet) on a 25,961-bp novel mosaic penicillinase plasmid (pPM1); MEStet was flanked by direct repeats of IS431, but with no target sequence repeats. Moreover, the PM1 genome was deficient in a copy of the restriction and modification genes (*hsdM* and *hsdS*), which might have contributed to the acquisition of enteroococcal multilac resistance.

**Citation:** Hung W-C, Takano T, Higuchi W, Iwao Y, Khokhlova O, et al. (2012) Comparative Genomics of Community-Acquired ST59 Methicillin-Resistant *Staphylococcus aureus* in Taiwan: Novel Mobile Resistance Structures with IS1216V. PLoS ONE 7(10): e46987. doi:10.1371/journal.pone.0046987

**Editor:** Michael Otto, National Institutes of Health, United States of America

**Received:** March 30, 2012; **Accepted** September 7, 2012; **Published** October 5, 2012

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**Funding:** No current external funding sources for this study.

**Competing Interests:** The authors have declared that no competing interests exist.

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**Introduction**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health problem worldwide. It was first isolated in 1961 as a nosocomial pathogen [1,2], now known as hospital-acquired MRSA (HA-MRSA). Another class of MRSA, designated community-acquired MRSA (CA-MRSA), emerged in the community from 1997 to 1999, posing a novel threat worldwide [1–4]. CA-MRSA infection usually occurs in children and young adults, or even the elderly, without exposure to hospital environments, and includes common skin and soft tissue infections (SSTIs) and occasionally life-threatening invasive infections, such as sepsis, necrotizing pneumonia and osteomyelitis [1–5].

CA-MRSA is characterized by the presence of staphylococcal cassette chromosome *mec* type IV (SCCmecIV) or SCCmecV [1,2,4,6]. SCCmecIV likely contributes to the spread in the community because it is smaller in size compared to other SCCmec elements [4,6,7] and has no effect on MRSA fitness (engendering no biological cost) [4,8]. CA-MRSA strains often produce Panton-Valentine leukocidin (PVL), which acts against neutrophils [4,9–11]. Moreover, CA-MRSA strains are only resistant to β-lactam antimicrobial agents or to some agents in restricted classes, although some recently successful CA-MRSA strains, such as multilocus sequence type 8 (ST8) CA-MRSA USA300, became multidrug-resistant [12,13]; USA300 has even spread into hospitals as a nosocomial pathogen [14,15].

A major CA-MRSA lineage in Taiwan is ST59 with SCCmecV and lukPVSF genes encoding PVL. Wang et al. [16] and Boyle-Vavra et al. [17] demonstrated that ST59 CA-MRSA in Taiwan was already resistant to multituds (including erythromycin, clindamycin, and chloramphenicol) when it began emerging in 1997, in contrast to the general understanding of CA-MRSA. Boyle-Vavra et al. [17] also characterized the SCCmec of Taiwanese ST59 CA-MRSA (representative strain, TSGH17) as SCCmec V. The ST59 CA-MRSA lineage (with SCCmec type V) and lukPVSF has also been reported in Singapore [18], Hong Kong [19], Japan [20,21], and Western Australia [22].

We further characterized the Taiwanese ST59 CA-MRSA lineage and found that it possessed a novel SCCmec with two distinct *cerG* genes (*cerG1* allele 2 and *cerG1* allele 8) [23–25]. We determined the entire SCCmec sequence of the ST59 CA-MRSA lineage (strain PM1) and tentatively designated it as SCCmecVII [24]; later, the SCCmec of the Taiwanese ST59 CA-MRSA lineage (strains TSGH17 and PM1) was reclassified as SCCmecV by the
International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements [6]. As for non-β-lactam resistance, three resistance determinants, embB (encoding erythromycin/clindamycin resistance), aph(3′)-IIIa (encoding kanamycin resistance), and adaE (encoding streptomycin resistance), were clustered in a region, previously named the drug resistance gene cluster (rgc) [23]. IS1216V is an enteroococal insertion sequence [26] and is rarely found in S. aureus. IS1216V-positive S. aureus cases include vancomycin-resistant MRSA (VRSA), in which IS1216V is inserted in a vancomycin resistance transposon (Tn1546) [27]. The 4g region of strain PM1 also carried IS1216V [23], raising the question of whether the 4g region of the Taiwanese ST59 CA-MRSA lineage (strain PM1) originated in enterococci through an IS1216V function.

In order to further understand the mechanism of multidrug resistance acquisition by PVL-positive ST39 CA-MRSA in Taiwan, we performed comparative genomics of strain PM1 and found that strain PM1 became multidrug resistant by acquiring two mobile genetic elements, an enteroococal IS1216V-mediated composite mobile element structure (MESPM1) on the chromosome and an IS43I-mediated element (MESad) on a plasmid, besides β-lactam resistance by SCCmecV. We also searched for the genetic background of the ST59 CA-MRSA lineage, which allowed (or stimulated) the acquisition of multidrug resistance.

**Results**

**Susceptibility to Non-β-lactam Agents of PVL-positive ST59/SCCmecV CA-MRSA Strains in Taiwan**

Drug resistance patterns of ST59/SCCmecV CA-MRSA strains isolated in Taiwan, compared to an ST59 MRSA-type strain USA1000, are summarized in Table 1. Half of the strains (including PM1) were resistant to erythromycin/clindamycin, kanamycin, streptomycin, chloramphenicol, and tetracycline, in addition to β-lactam agents, showing multidrug resistance (in contrast to USA1000). The rest showed five distinct patterns in terms of resistance to non-β-lactam agents: resistance to erythromycin/clindamycin, kanamycin, streptomycin, and chloramphenicol; resistance to erythromycin/clindamycin, kanamycin, streptomycin, and tetracycline; resistance to erythromycin/clindamycin, kanamycin, and streptomycin; resistance to chloramphenicol and tetracycline; and non-resistance (to any non-β-lactam agent).

**Comparative Genomics of PVL-positive Multidrug-resistant ST59/SCCmecV CA-MRSA (Strain PM1)**

The PM1 genome was analyzed by pyrosequencing (GenBank Accession number BAFA01000000) and compared with the genomes of ST398 MRSA human strain S0385 and PVL-positive ST8 CA-MRSA USA300, since ST398 MRSA carried SCCmecV [28], similar to PM1, and showed the highest similarity to ST59 MRSA [29] in terms of the seven housekeeping gene sequences used for multilocus sequence typing, and USA300 was one of the best characterized CA-MRSA [30]. The data are summarized in Figure 1. The alignment of PM1 contigs on the reference MRSA genome was the same in both cases (Figure 1A); the similarities of the PM1 genome to the ST398 and ST8 genomes were both approximately 98%, albeit with divergence in, for example, the carriage of mobile elements (Figure 1A). The PM1 genome was estimated to be at least 2.8 Mb. Strain PM1 carried a 25,961-bp plasmid (pPM1), encoding tetracycline resistance; plasmid DNA analysis of strain PM1 also demonstrated a single species of plasmid of 26.0 kb (Table 1).

When PM1 was compared with S0385 and USA300, the four regions were unique to PM1: i) SCCmecV with two ccrC genes (ccrC1 allele 2 and ccrC1 allele 8); ii) φSA1PM1, which was 70.2% homologous to φETA3, a member of the φSA1 family (φETA3 is ete-positive, while φSA1PM1 was negative); iii) MESPM1, a novel mobile element structure on the chromosome, encoding multidrug resistance; and iv) a tetracycline resistance plasmid pPM1. Moreover, vSAβ on the PM1 genome had a large deletion.

**Structure of MESPM1**

The entire sequence of MESPM1 and its surrounding region was determined (GenBank Accession number AB699882); the structure is summarized in Figure 2. MESPM1 was 21,832 bp long and flanked by direct repeats of IS1216V at both ends. It was inserted into the att site (8-bp target sequence) within the sasK gene; the 8-bp att sequence was duplicated at both ends of MESPM1, indicating that MESPM1 is a large transposon. The target gene (sasK) with the 8-bp att sequence was present on the genome of ST59 MRSA USA1000, but not in strains S0385 and USA300 (Figure 1A). Interestingly, MESPM1 was IS1216V-rich and contained three additional copies of IS1216V (a total of five copies of IS1216V), as shown Figure 2.

**Table 1. Resistance patterns of ST59 CA-MRSA strains isolated in Taiwan, compared to ST59 MRSA USA1000.**

| Resistance patterns* | Percentage (no./total) | Example (strain) | Plasmidb (size) |
|----------------------|------------------------|-----------------|-----------------|
| Ery/Cli Kan Stm Chl Tet |                        |                 |                 |
| R        R        R        R      R | 50% (12/24)         | PM1, PM22       | 26 kb           |
| R        R        R        R      S | 16.7% (4/24)        | PM11            | 21 kb           |
| R        R        S        R      S | 16.7% (4/24)        | PM9             | 26 kb           |
| R        R        S        S      S | 8.3% (2/24)         | PM12            | None            |
| S        S        S        R      S | 4.2% (1/24)         | PM8             | 26 kb           |
| S        S        S        S      S | 4.2% (1/24)         | PM18            | 21 kb           |
| R(Ery)   S        S        S      S |                        | USA1000         | ND*             |

*The number of strains tested was 24. Non-β-lactam agents: Ery, erythromycin; Cli, clindamycin; Kan, kanamycin; Stm, streptomycin; Chl, chloramphenicol; Tet, tetracycline. USA1000 is a type strain of PVL-positive ST59 MRSA.

bPlasmid analysis was carried out for example strains.

*Not determined.

doi:10.1371/journal.pone.0046987.t001
Genome and Resistance of Taiwanese ST59 CA-MRSA

A

Ery/Cli', Kan', Stm', Chl'

Oxa'

MES_{PM1}

SCCmecV (ccrC1 alleles 2 and 8)

B

Relevant genotype of strain PM1

| Genotype | ST59/spa143(t437)/agr1a/SCCmecV/CoaVII |
|-----------------|----------------------------------------|
| Drug resistance | ermB (Ery/Cli), aph(3')-IIIla (Kan), aadE (Stm), cat (Chl), tetK (Tet) |
| Virulence factor | lukP_{PV}SF (PVL) |
| Leukocidin | hla, hib, hlg, hlg-v, hld |
| Hemolysin | seb, sek, seq |
| Enterotoxin | icaA, icaD (for biofilm formation); eno (for laminin-adhesin); fnbA, fnbB (for fibronectin-adhesin); ebpS (for elastin-adhesin); clfA, clfB, fib, sdrC, sdrE (for fibrinogen-adhesin) |
| Adhesin | |
| Phage | φSA_{1, pm1}, φSA_{2, pm1} (lukP_{PV}SF) |
| Pathogenicity island | SaPI3 (seb, sek, seq) |
| Mobile element | MES_{PM1} (ermB, aph[3']-IIIla, aadE, cat), MES_{int} (tetK) |
| Transposon | Tn551 (ermB), Tn5405-like (aph[3']-IIIla, aadE), Tn552-like (bla) |
| Insertion sequence | IS232-like, IS431, IS1216V, IS1272, IS200-like family |
The left side of MESPM1 was a composite transposon region consisting of Tn551 carrying emB and Tn5405-like carrying aph(3′)-IIIa and aadE. The composite transposon region was identical to the corresponding region of a plasmid (pLG2) of Enterococcus faecalis (Figure 2); the entire sequence of pLG2 has not been reported.

Analysis of the right-side region of MESPM1 revealed an independent transposon (MEScat) carrying cat (encoding chloramphenicol resistance). MEScat was 6,587 bp long and flanked by the inverted repeats of IS1216V; moreover, the 8-bp att sequence was duplicated at both ends of MEScat (Figure 2). MEScat contained two additional copies of IS1216V. The central cat region in MEScat was highly homologous (99.5%) to the cat region of SAP084A plasmid in S. aureus.

Based on the data, we conclude that MESPM1 is a highly composite transposon, which originated in enterococci, but acquired an additional drug resistance gene (cat) in S. aureus. Another multidrug-resistant strain PM22 (Table 1) also possessed an MES structure (MESPM2) very similar to MESPM1, although there was a small deletion within the structure (data not shown).

Structure of MESPM1 Segregants with Different Resistance Patterns

The MESPM1-corresponding region of ST59 CA-MRSA strains with different resistance patterns (Table 1) was determined by sequencing (Figure 3). MESPM1 seems genetically unstable, and recombination likely takes place between two direct repeats of IS1216V, resulting in deletion, which could explain the distinct resistance patterns of ST59 CA-MRSA.

For instance, strain PM9, which was resistant to erythromycin/clindamycin, kanamycin, and streptomycin, but susceptible to chloramphenicol, seemed to be a segregant, generated through deletion (1) in Figure 3 (A, B). Likewise, strain PM8, which was resistant to chloramphenicol only, seemed to be a segregant, generated through deletion (2) in Figure 3 (A, C). Strain PM18, which was susceptible to all the non-β-lactam agents, seemed to be a segregant, generated through deletion (3) in Figure 3 (A, D).

MESPM1 segregants, MESPM10 (Figure 3B) and MESPM11 (Figure 3C), may also behave as transposons, since they were flanked by direct repeats of IS1216V at both ends with att sequence duplication and inserted into the sasK gene, just like MESPM1. MESPM11 (corresponding to IS1216V), which was inserted into the

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**Figure 1.** Genome comparisons of ST59 MRSA strain PM1 with two other S. aureus genomes. In A, two gray circles show the complete genomes of ST8 CA-MRSA strain USA300 (2,872,769 bp) and ST398 MRSA strain S0385 (2,872,582 bp); gaps in the genome circles indicate USA300 or S0385 sequences not present in PM1. The sasK gene with the MESPM1 integration site (att) is shown outside the gray circles (on the USA1000 genome circle), since the USA300 and S0385 MRSA strains do not possess the sasK gene. The outermost (white) circle represents the alignment of PM1 contigs and provides PM1 genome information, including drug resistance traits, pathogenetic islands, bacteriophages, and genomic islands. Color region in the genome: orange, orfX with the att sequence for SCCmec; green, genetic elements with diversity; brown, genetic elements present in USA300 or S0385 (but not in PM1); pink, genetic elements present in PM1 (but not in USA300 or S0385). In B, relevant genotypes of PM1 is summarized. Drug resistance (r): Ery/Cli, erythromycin/clindamycin; Kan, kanamycin; Stm, streptomycin; Chl, chloramphenicol; Oxa, oxacillin; Tet, tetracycline. Virulence factors: SEB, staphylococcal enterotoxin B; SEK, staphylococcal enterotoxin K; SEQ, staphylococcal enterotoxin Q; PVL, Panton-Valentine leukocidin.

doi:10.1371/journal.pone.0046987.g001

**Figure 2.** Genetic structure of MESPM1 carrying four resistance genes, emB, aph(3′)-IIIa, aadE, and cat. MESPM1 was integrated into and disrupted the sasK gene, which encoded a putative cell-wall anchored surface protein with the LPXTG-motif. The intact sasK gene was present in PVL-positive ST59 CA-MRSA type strain USA1000. Three att sites are in a vertical red line. The region of IS1216V with the transposase gene (tnp) is shaded in blue. Homology regions with E. faecalis pLG2 plasmid (GenBank accession number HQ426665) and S. aureus SAP084A (GenBank accession number GQ900436) are shaded in yellow and purple, respectively. A Greek delta symbol indicates truncated coding sequence.

doi:10.1371/journal.pone.0046987.g002
A  **$\text{MES}_{PM1}$ in PM1 (Ery/Cli\(^r\), Kan\(^r\), Stm\(^r\), Chl\(^r\))**

![Diagram of MES\(_{PM1}\) in PM1](image)

B  **$\text{MES}_{PM9}$ in PM9 (Ery/Cli\(^r\), Kan\(^r\), Stm\(^r\))**

![Diagram of MES\(_{PM9}\) in PM9](image)

C  **$\text{MES}_{PM8}$ in PM8 (Chl\(^r\))**

![Diagram of MES\(_{PM8}\) in PM8](image)

D  **$\text{MES}_{PM18}$ (IS\(_{1216V}\)) in PM18**

![Diagram of MES\(_{PM18}\) (IS\(_{1216V}\))](image)
sasK gene and possessed att sequence duplication at both ends (Figure 3D), may behave as an IS.

**Structure of pPM1**

The entire sequence of pPM1 was determined (GenBank Accession number AB699881) and its structure is summarized in Figure 4A. pPM1 was a novel composite plasmid, consisting of a replication (rep) region (homologous to pSK156 plasmid), cadmium resistance (cad) region (homologous to SAP063A plasmid), ampicillin resistance (bla) region (homologous to SAP063A plasmid), and tetracycline resistance (tetK) region (homologous to SAP014A plasmid). The tetK-carrying region was 6,025 bp long and was flanked by direct repeats of IS\textsubscript{431} at both ends; this structure was designated MES\textsubscript{tet}. There was no att sequence duplication at either end of MES\textsubscript{tet}.

**Analysis of Tetracycline-susceptible Strains**

Of three tetracycline-susceptible strains (Table 1) examined, two strains, PM11 and PM18, carried a pPM1-related plasmid (pPM11 and pPM18, respectively). Entire sequence analysis revealed that the two plasmids carried one copy of IS\textsubscript{431} only at the MES\textsubscript{tet} insertion site on pPM1, as shown in Figure 4B; therefore, pPM11 and pPM18 could be a segregant of pPM1 generated through a recombination/deletion event between two direct repeats of IS\textsubscript{431}. The remaining strain (PM12) lacked pPM1 plasmid.

**Conjugative Transfer of Drug Resistance**

Conjugative transfer of drug resistance was performed between PM1 (donor strain) and \textit{S. aureus} RN2677 (recipient strain) by filter mating. As shown in Table 2, resistance to ampicillin, tetracycline, and cadmium was transferred to \textit{S. aureus} RN2677 with equal frequency; all transconjugants obtained carried a 26-kp penicillinase plasmid (pPM1), as expected. However, no MES\textsubscript{PM1}-associated transconjugants (exhibiting resistance to erythromycin, clindamycin, chloramphenicol or streptomycin) were obtained.

**Restriction and Modification Genes (hsdS and hsdM) in Strain PM1**

Genomic island vSA\textsubscript{b} of MRSA strains, e.g. USA300, carried intact hsdS and hsdM, while vSA\textsubscript{b} of PM1 carried truncated hsdS and lacked hsdM (Figure 5), although genomic island vSA\textsubscript{a} of PM1 carried an intact set of hsdS and hsdM, similar to USA300 (Figure 1). Lack of a set of hsdS and hsdM in PM1 may have contributed, in part, to PM1’s potential to acquire foreign DNA.

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**Figure 3. The structure of MES\textsubscript{PM1} and its segregants in ST59 CA-MRSA strains.** Structures of MES\textsubscript{PM1}, MES\textsubscript{PM9}, MES\textsubscript{PM8}, and MES\textsubscript{PM18} (corresponding to IS\textsubscript{1216V}) are shown in A to D. Drug resistance (r): Ery/Cli, erythromycin/clindamycin; Kan, kanamycin; Stm, streptomycin; Chl, chloramphenicol. The IS\textsubscript{1216V} region with the transposase gene (tnp) is shaded in blue. The att site is indicated by a vertical red line. Arrows below the MES structures indicate PCR primers, which are listed in Materials and Methods.

doi:10.1371/journal.pone.0046987.g003

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**Figure 4. Genetic structure of penicillinase plasmid pPM1 and its segregant.** In A, pPM1 structure is shown. Inner circle indicates homologous regions to \textit{S. aureus} plasmid SAP014A (GenBank accession number GQ900379), SAP063A (GenBank accession number GQ900418), and pSK156 (GenBank accession number GQ900448). The IS\textsubscript{431} region with the transposase gene (tnp) is shaded in light green. A Greek delta symbol indicates truncated coding sequence. In B, a segregant of pPM1 in MES\textsubscript{tet} locus is shown. Tet\textsuperscript{r}, tetracycline-resistant; Tet\textsuperscript{s}, tetracycline-susceptible.

doi:10.1371/journal.pone.0046987.g004
Discussion

In this study, we performed comparative genomic analysis of a multidrug-resistant, PVL-positive ST59/SCCmeV CA-MRSA strain (PM1) and found MESPM1 on the PM1 chromosome, encoding resistance to erythromycin, clindamycin, kanamycin, streptomycin, and chloramphenicol. As shown in Figure 6A, MESPM1 exhibited typical features of a transposon, such as the presence of terminal repeats with 8-bp att duplications at both ends. The unique features of MESPM1 include the presence of IS1216V as a terminal direct repeat and a composite transposon region containing Tn551 and Tn5405-like, both of which...
originate in enterococci (such as *E. faecium* and *E. faecalis*) [26,33,34], strongly suggesting the origin of MESPM1 in enterococci.

In *E. faecium*, IS1216V is considered to be part of a large mobile element containing Tn1546 with *vanA* (encoding vancomycin resistance) [35], as shown in Figure 6B (a); however, a possible mobile structure with *att* duplication has not been reported. Moreover, in *E. faecium*, a large mobile element (Tn5506), which contains Tn1546 and is flanked by hetero terminal sequences, IS1216V and IS1252, with 8-bp *att* duplications at both ends, has been described [32], as shown in Figure 6B (b); however, the *att* of Tn5506 was different from the *att* of MESPM1 (Figure 6A and B [b]), and in the case of Tn5506, another copy of IS1216V was located adjacent to IS1252 (Figure 6B [b]).

Transfer of the *van* genes (such as *vanA*) from enterococci to MRSA would be a great public health concern and threat, because
vancomycin is the first-line agent for MRSA infections [3,6,37]. Unfortunately, Tn1546 has already emerged in MRSA [27], as shown in Figure 6G; in this case, IS1216V is present near the _vanA_ genes (although possible IS1216V repeats and _att_ duplication have not been reported).

Therefore, this study demonstrates the first IS1216V-mediated mobile element structure with terminal direct repeats of IS1216V with _att_ duplication at both ends. Interestingly, MESPM1 also contained another mobile element structure (MES_81) within the structure, which was flanked by _att_ 1216V at both ends. Chloramphenicol resistance in MRSA is usually encoded by a plasmid [31,38]; however, in this case, it was encoded by MES_81 (as a part of MESPM1) on the chromosome. The chloramphenicol resistance region seemed to originate in _S. aureus_; therefore, MESPM1 is a highly composite structure with mobile elements originating in _Enterococcus_ and _Staphylococcus_ species.

This study clearly concludes that the acquisition mechanism of multidrug resistance in ST59 CA-MRSA in Taiwan is quite different from many other MRSA strains, but shows some similarity to VRSA cases, and that the MESPM1 acquisition event occurred most probably around 2007 or earlier in Taiwan, and then ST59 CA-MRSA carrying MESPM1 spread in Taiwan, followed by segregation events generating different resistance patterns.

The reason why ST59 CA-MRSA in Taiwan acquired MESPM1 is not known. The type I restriction-modification system, composed of _HsdR_ (restriction), _HsdM_ (modification), and _HsdS_ (sequence specificity), is known to block horizontal gene transfer from other species into _S. aureus_, as well as to limit DNA exchange between the different lineages of _S. aureus_ [39,40]. Deficiency in the type I restriction-modification system would allow foreign DNA acquisition with high frequency. For example, a bovine pathogenic _S. aureus_ strain, which is naturally mutated in both _hsdS_ genes, has been reported displaying a “hyper-recipient” phenotype to acquire DNA from _Enterococcus_ species [41]. In the case of ST59 CA-MRSA (strain PM1) in Taiwan, a copy of _hsdM_ and _hsdS_ in _vsaB_ was truncated, which might contribute to the acquisition of foreign DNA from enterococci such as IS1216V and MESPM1; further study is needed for elucidation.

In this study, a conjugative penicillinase plasmid (pPM1) in strain PM1 was successfully transferred by filter mating, while MESPM1 was not, suggesting that MESPM1 may not be a conjugative transposon.

In strain PM1, we also identified a novel mosaic penicillinase plasmid encoding tetracycline resistance (pPM1). The tetracycline resistance region (MES_81) could be a novel transposon flanked by IS431, which most probably originated in _S. aureus_. This structure also generated segregants (tetracycline-susceptible penicillinase plasmid) resulting in strains PM11 and PM18.

Regarding other genetic structures in ST59 CA-MRSA in Taiwan, we confirmed the sequence of SCCmecV of strain PM1 (formerly SCCmecVII) [24] and the sequence of PVL phage (φ3967PLC; φSA2PM1 in this study), which was originally reported with another strain (JCSC5967) by Zhang et al. [42]; they were 99.9% homologous.

During the course of this study, Huang et al. described the genome sequence of ST59 CA-MRSA strain M013 in Taiwan [43], although they did not describe the plasmid(s). According to their data, their strain (M013) is not multidrug-resistant; most probably it corresponds to strain PM18 in this study, a minor derivative of PM1 carrying only IS1216V at the MESPM1 insertion site and showing no resistance to non-β-lactam agents. M013 also does not carry φSA1PM1. In addition, three genes ( _sasA_ , _fikA_ , and _sdcB_ ) in M013 have more than 24-bp deletions or insertions. The comparison between the PM1 and M013 genomes is summarized in Table S1.

In conclusion, an IS1216V-mediated mobile element structure (MESPM1), encoding multidrug resistance and originating in enterococci, has emerged in PVL-positive ST59 CA-MRSA (e.g. strain PM1) in Taiwan. Although IS1216V, related to vancomycin resistance transposon Tn1546, has been reported in MRSA, MESPM1 represents the first mobile element flanked by IS1216V direct repeats with _att_ duplication at both ends. MESPM1 also includes additional _HsdS_ flanked by IS1216V inverted repeats with _att_ duplication at both ends, although the _cat_ region originated in _S. aureus_ indicating a highly composite formation in its evolution. The deficiency of a copy of _hsdM_ and _hsdS_ (in _vsaB_ ) in strain PM1 might contribute to the acquisition of foreign DNA, such as IS1216V and MESPM1, from enterococci.

### Materials and Methods

#### Bacterial Strains

Twenty-four PVL-positive ST59/SCCmecV CA-MRSA strains were collected from 2000 to 2006 in the Bacteriology Laboratory, National Taiwan University Hospital. These strains were isolated from patients with SSTI, bacteremia, respiratory tract infection, and surgical wounds [23]. USA1000 is an ST59 MRSA-type strain (of the Smal PFE type) isolated in the United States, and was kindly provided by L. K. Dougal and L.L. McDonald.

#### Pyrosequencing Analysis of the Genome

The PM1 genome was analyzed by pyrosequencing using a genome sequencer FLX system (Roche Diagnostics, Branford, CT, USA). It yielded 93 Mb raw sequences by 236,943 reads, corresponding to approximately 33.2-fold of the genome size. Contigs were then assembled according to the two genomes of ST8 MRSA USA300 FPR3757 (GenBank accession number NC_007793) and ST398 MRSA human strain S0385 (GenBank accession number AM999992). Open reading frames were searched for using the software in silico MolecularCloning (version 4.2) (In Silico Biology, Yokohama, Japan) or DNAman software package (Version 6) (Lynnon Biosoft, Quebec, Canada).

#### Entire Sequencing of Mobile Elements and a Plasmid

The gaps between contigs, related to mobile elements, were filled by PCR and sequencing. We also assembled contigs using an LA PCR in vitro cloning kit (Takara Bio, Otsu, Japan) according to the manufacturer’s instructions. In brief, after digestion with suitable restriction enzymes and ligation with the corresponding cassette adapters, amplification was performed with cassette primers and target-specific primers.

#### PCR Analysis of the MESPM1 Structure in Other PVL-positive ST59 CA-MRSA Strains

Based on the determined entire sequence of MESPM1, a series of primer sets was designed (Table 3) and used to screen the genetic constitution of MESPM1. In PCR reactions, 10% glycerol or 4% DMSO was added in order to dissolve the secondary structure of IS1216V.

#### Susceptibility Testing

Antimicrobial susceptibility tests were performed by the agar dilution method with Mueller-Hinton agar (Difco, Sparks, MD, USA) according to the guidelines of the Clinical and Laboratory Standards Institute [44].
Conjugative Transfer

Donor strains were mated with S. aureus RN2677 (recipient strain, which is restriction-negative and resistant to rifampicin and novobiocin) on membrane filters on tryptic soy agar (Difco) (filter mating), as previously described [45]; RN2677 was used as a recipient because it carried no plasmids and had non-transmissible drug resistance (recipient) markers. The resistance genes of transconjugants were examined by PCR as previously described [23].

Plasmid DNA

Plasmid DNA was isolated using a plasmid midi kit (Qiagen, Hilden, Germany) and lysostaphin (Wako Pure Chemicals, Osaka, Japan), following the manufacturer’s instructions.

Supporting Information

Table S1 Comparative genomic analysis between PM1 and M013.

(XLS)

Acknowledgments

We thank Akihito Nishiyama for technique assistance and discussion, and L. K. McDougall and L. L. McDonald for USA1000, an ST59 MRSA-type strain.

Author Contributions

Conceived and designed the experiments: TY WCH TT. Performed the experiments: TY HCH TT WH YI. Analyzed the data: TY HCH TT WH YI. Contributed reagents/materials/analysis tools: WCH TT WH YI. OK. Wrote the paper: TY WCH.

Table 3. Primers used for PCR mapping of the MESPM1 locus.

| Primer name | Sequence (5’ to 3’) |
|-------------|---------------------|
| 11F2        | AGGCCAACATTTGGAAGT  |
| ermBR       | GCAATGAAACACGCC    |
| ermB        | AGTAAACGGTACTAAATGTTTC  |
| aadE        | ACGGTCAATACATTGATGG  |
| 1F          | AGTAGCCTTCCTTCATCT    |
| 3SR         | CTCGGTACGGTACGGCA    |
| 35F         | CTTACCAGGGTTCGGA    |
| LA-R2       | CCCATGCAGGTTTCAAAATGTGTAAGTCA  |
| LA-F1       | ACACCAGAACAATGTAATCAACTAGGGT |
| 23R         | TGTTCCAAAGGCCATCA    |
| 23F         | CACTAGGAGGCGCAAGAA   |
| 48R3        | TCAGGCTGGAATAATAGA    |
| catR        | GCATTTGATTGTCTCAGCA  |

doi:10.1371/journal.pone.0046987.t003

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