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MOLECULAR CHARACTERIZATION OF METHICILIN-RESISTANT *Staphylococcus aureus* STRAIN BM85 ISOLATED FROM A VIETNAMESE PATIENT WITH BLOODSTREAM INFECTION

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a threat to global health due to its resistance to β-lactam antibiotics and many other antibiotic classes developed via both intrinsic and acquired mechanisms. In this study, molecular characteristics related to antibiotic resistance of MRSA strain BM85 were investigated by whole-genome sequencing of a sample isolated from a patient with bloodstream infection at Bach Mai Hospital, Vietnam. Antibiotic susceptibility testing was also performed to determine the correlation between the presence of antibiotic-resistant genes and resistance phenotypes. Genomic analyses showed that the MRSA strain BM85 belonged to the major community-acquired (CA)-MRSA lineage ST59 originating from Taiwan. The strain harbored Staphylococcal Cassette Chromosome mec (SCCmec) type Vb (5C2&5) and Panton-Valentine leukocidin (PVL). Additionally, MRSA strain BM85 also possessed various antibiotic-resistant genes including *tet*38, *tet*K, *bla*Z, *mec*A, *aph*(3')-IIIa, *aac*A-*aph*D, and *erm*B which were located on mobile genetic elements MESPM1, Tn553-Tn4001 transposon, and a plasmid carrying the *tet*K gene, which was responsible for tetracycline resistance. The genotypic-resistant results were concordant with the phenotypic-resistant profile in which MRSA strain BM85 was resistant to penicillin, cefoxitin, gentamicin, kanamycin, tobramycin, erythromycin, and tetracycline. The sequencing data for the MRSA strain BM85 was deposited in GenBank, NCBI under accession number: BioProject PRJNA857185. In conclusion, the acquisition of the foreign genetic elements associated with antibiotic resistances through horizontal gene transfer mechanisms was the key driver of multidrug resistance in the multidrug-resistant MRSA strain BM85.

Keywords: Methicillin-resistant *Staphylococcus aureus*, antibiotic resistance genes, multidrug resistance, bioinformatics, whole-genome sequencing.

INTRODUCTION

Staphylococcus aureus is a significant opportunistic pathogen in humans associated with hospital-acquired (HA) or community-acquired (CA) infections. Approximately 20-30% of the human population is colonized with the bacteria which poses risks for potential

infections (Belkum *et al*., 2009; Wertheim *et al.*, 2005). The treatment for *S. aureus* infections has been challenging due to the remarkable ability to acquire resistance to multiple antibiotic classes. The emergence of antibiotic-resistant *S. aureus* occurred through a series of endemic and epidemic waves over the past 80 years (Graves, Kobayashi, DeLeo, 2010; Guo, Song, Sun, Wang, Wang, 2020). The methicillin-resistant *S. aureus* (MRSA) was first reported in 1961 as a nosocomial pathogen and the emergence of new MRSA strains over the last decades posed the increased burden of antibiotic resistance in both hospitals and community settings. MRSA is resistant to broad-spectrum β-lactam antibiotics via the acquisition of a genetic structure called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). The SCC*mec* is a mobile element with a length of 20 to 67 kilobase (kb) carrying resistance-determinant gene *mec*A or *mec*C which encodes for penicillin-binding protein 2a (PBP2a) (Hiramatsu *et al.*, 2013). PBP2a has a low affinity to beta-lactams therefore it can take over the cell wall biosynthesis while other PBPs are inactivated in the presence of beta-lactams (Hanssen, Ericson Sollid, 2006). Due to the difficulties in treatment, MRSA was widely disseminated and has become a major public health problem worldwide.

In Vietnam, the MDR rate of HA-MRSA strains isolated from children from the two biggest tertiary care pediatric hospitals in northern and southern Vietnam was reported to be 51.8% (Nguyen *et al.*, 2019). Other studies on MRSA strains isolated in adults from four tertiary hospitals in three regions of Vietnam showed an even higher co-resistant rate to three or more non-β-lactam antimicrobial classes of 72% (Nguyen *et al.*, 2020; Vu *et al.*, 2016). Moreover, the study of Nguyen *et al*., 2020 also revealed geographical differences with a substantial increasing trend from north to central and to south in terms of antibiotic resistance profile, MDR rates for MRSA isolates. These findings were limited to big tertiary hospitals and not on a national scale, however, they showed the high occurrence of MDR-MRSA with the highest co-resistance rate to four distinct antibiotics classes other than β-lactam, which confirmed the great challenges to controlling and treating *S. aureus* infections in Vietnam.

Whole-genome sequencing (WGS) and computational methods have complemented traditional culture-based, phenotypic susceptibility testing by enabling rapid and sensitive identification of antimicrobial resistance determinants (Koser, Ellington, Peacock, 2014). Moreover, WGS has been used to study the mechanism of antibiotic resistance while providing new insights. A number of studies have shown the transfer of *mec*A gene between *S. aureus* and *Staphylococcus epidermidis* (Bloemendaal, Brouwer, Fluit, 2010). Similarly, by applying sequencing technologies Kos *et al.* proved that vancomycin-resistant *S. aureus* strains acquired resistance genes from enterococci (Kos *et al.*, 2012). In the context of MRSA investigations, it has been shown that antibiotic susceptibility phenotypes can be reliably predicted by interrogating WGS data *in silico* (Koser *et al.*, 2012). However, the ability to predict phenotypes from genetic information depends on a thorough knowledge of resistance mechanisms. It is necessary to closely evaluate the relationship between the resistance defined by WGS and current standard phenotypic methods because the presence or absence of a resistance gene does not always correlate with resistance. Collectively, the combination of both *in vitro* antibiotic resistance testing and *in silico* genomic analyses can provide comprehensive understandings of not only the correlation between antibiotic-resistant genotype and phenotype profiles but also the underlying genetic mechanisms contributing to the evolution and development of MDR bacteria. In this context, a multidrug-resistant MRSA strain BM85 isolated from a Vietnamese patient with bloodstream infection was characterized by performing both *in vitro* antibiotic resistance testing and *in silico* genomic analyses using the WGS technology.

MATERIALS AND METHODS

Bacterial strain

The methicillin-resistant *S. aureus* strain BM85 (MRSA strain BM85) was provided by the National Institute of Hygiene and Epidemiology (NIHE). This strain was previously isolated from a bloodstream-infected patient in Bach Mai Hospital in 2015 and was identified as *S. aureus* species using a MALDI-TOF mass spectrometry system at the hospital. The strain was determined as methicillin resistance using a Vitek-2 compact system.

Antibiotic susceptibility testing

The antibiotic susceptibility testing for MRSA strain BM85 was performed by disk diffusion method. The selected antibiotics included penicillin (1 IU), cefoxitin (30 µg), gentamicin (10 µg), kanamycin (30 µg), tobramicin (10 µg), rifampin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), fosfomycin (200 µg), erythromycin (15 µg), ofloxacin (5 µg), fusidic acid (10 µg), pristinamycin (15 µg), and tetracycline (30 µg). The antibiotic susceptibility results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

DNA extraction and whole-genome sequencing

Genomic DNA of MRSA strain BM85 was extracted using Bacterial Genomic DNA Isolation Kit (Norgen Biotek Corp., Thorold, Ontario, Canada) according to the manufacturer's protocol and the quality of extracted genomic DNA was evaluated using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The genome of MRSA strain BM85 was subsequently sequenced using an Illumina HiSeq 4000 system (Illumina, San Diego, CA, USA) at the Beijing Genomics Institute (Shenzhen, China). A total of 8,764,210 150-bp paired-end reads were generated.

De novo assembly and genome annotation

Raw sequencing data were first assessed for quality control using FastQC, then trimmed and quality-filtered by Trimmomatic (Bolger, Lohse, Usadel, 2014). SPAdes was used with default parameters for *de novo* assembly (Bankevich *et al.*, 2012). The assembly metrics were generated by QUAST for evaluating and comparing with or without *S. aureus* NCTC 8523 (GenBank accession number NC007795) as the reference genome (Gurevich, Saveliev, Vyahhi, Tesler, 2013). Assembled Contigs were then annotated using the web service Rapid Annotations using Subsystems Technology (RAST; https://rast.nmpdr.org/), and PATRIC 3.6.12 (https://www.patricbrc.org/).

Whole-genome sequence analysis

Multiple-genomic alignment with contig rearrangement and map visualizations were performed using Mauve software (Darling, Mau, Blattner, Perna, 2004) and BLAST Ring Image Generator (BRIG) software (Alikhan, Petty, Ben Zakour, & Beatson, 2011), respectively. *In silico* Staphylococcal cassette chromosome *mec* (SCC*mec*) typing, conventional seven-gene multi-locus sequence typing (MLST), staphylococcal protein A (*spa*) typing, and virulence factor identification were performed using web-based SCC*mec*Finder (Kaya *et al.*, 2018), MLST 2.0 (Larsen *et al.*, 2012), and *spa*Typer (Bartels *et al.*, 2014), respectively, available online at Center for Genomic Epidemiology server (http://www.genomicepidemiology.org/). For *in silico* coagulase gene typing, the full amino acid sequence of coagulase was extracted from RAST-annotation output and searched against the non-redundant protein sequence database of NCBI using BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). Accessory gene regulator (*agr*) typing for BM85 was also conducted *in silico* by aligning the full-length nucleotide sequence of *agr* gene to the curated variable nucleotide region of four *agr* types described previously by using BLASTP (Higuchi *et al.*, 2010). Virulence factors were predicted using the online tool VFanalyzer (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi). Phage regions in BM85

genome were identified by PHAge Search Tool Enhanced Release server (PHASTER; http://phaster.ca/). Genomic islands (GIs) were predicted using IslandViewer4 (https://www.pathogenomics.sfu.ca/islandviewer/). All genomic predictions and identifications were accomplished with default parameters, confirmed by manual contigs reconstruction, re-annotation, cross-examination with RAST outputs, and validated by BLAST programs.

Antimicrobial resistance (AMR) gene identification

Resistance Genes Identifier (RGI; https://card.mcmaster.ca/analyze/rgi) was used to preliminarily identify AMR genes in the BM85 draft genome against the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/). Resistance-coferring mutations predicted by RGI were manually inspected with reference to the corresponding literature publications provided by CARD for verification using BioEdit. The chromosomally-encoded multidrug-resistant (MDR) efflux pumps of the strain BM85 were characterized by (Costa, Viveiros, Amaral, & Couto, 2013). All predicted AMR genes were cross-examined with RAST- and PATRIC-annotation outputs, and manually validated using BLAST programs, information reviewed in the Transporter Classification Database (TCDB) and WikiGenes (https://www.wikigenes.org/).

RESULTS AND DISCUSSION

***De novo* assembly and general features of BM85 draft genome**

General features of the draft genome of MRSA strain BM85 are shown in Table 1. The draft genome sequence of strain BM85 consisted of 95 contigs, comprising 2,797,918 bases with the overall G+C content being 32.7%. The RAST server predicted a total of 2,655 coding sequences and 71 RNA genes. Notably, among 275 subsystems, there were 4 replication initiation proteins grouped in the plasmid-related rolling-circle replication subsystem, suggesting that MRSA strain BM85 was likely to carry plasmids or mobile genetic elements originating from plasmids on its chromosome.

Table 1. General features of MRSA strain BM85 draft genome.

Characteristics	Value
Genome size (bp)	2,797,918
GC Content (%)	32.7
Number of Contigs	95
N50 (bp)	122,702
L50	7
Number of Coding Sequences	2,655
Number of RNAs	71
Number of Subsystems	275

N50: the sequence length of the shortest contig at 50 % of the total assembly length; L50: the smallest number of contigs whose length sum makes up half of the assembly size.

MRSA strain BM85 belonged to the Taiwan MRSA clone carrying multiple virulence and antibiotic-resistance genes

Analysis of Multilocus Sequence Typing (MLST) showed that the MRSA strain BM85 belonged to sequence type 59 (ST59) which is an epidemic lineage of community-associated methicillin-resistant *S. aureus* (CA-MRSA) in Asia (Song et al., 2011). Analysis of genotyping and virulence factor identified SCC*mec* Vb (5C2&5), and $luk_{PV}SF$ genes encoding Panton-Valentine leukocidin (PVL) which is similar to CA-MRSA strain M013's genotype (Table 2 and Figure 1). The ST59/SCC*mec*Vb/*pvl*-positive genotype is the typical genotype for a major Taiwan CA-MRSA lineage internationally known as Taiwan clone, suggesting that MRSA strain BM85 was likely derived from the Taiwan clone (Higuchi et al., 2010). To further elucidate the genomic characteristics of MRSA strain BM85, CA-MRSA strain PM1, a Taiwan clone whose genomic features were well-

characterized, was chosen for comparative genomic analysis, along with the previously selected strain M013. Other aspects of genotype including accessory gene regulator (*agr*) type, staphylococcal protein A (*spa*) type, and coagulase (Coa) type were exhibited in strain PM1 as *agr*1a, t437, and CoaVII, respectively, were identically identified *in silico* in MRSA strain BM85 (Figure 1). Moreover, genomic analysis of BM85 strains BM85 and PM1 with the reference genome of strain M013 showed that they had high genomic similarity and shared a common virulence gene profile, indicating similar pathogenic characteristics (Figure 1).

Table 2. Genomic features of MRSA strain BM85.

Genotype	
Sequence type (ST)	59
SCC*mec* type	Vb (5C2&5)
spa	143 (t437)
agr	1a†
Coagulase type	VII
Plasmid	pM013
Genomic island	vSAα (*sslś*), vSAβ (*scn, chp*), vSAγ (*ssls, hla, scn, efb, ecb, flpr-1**)
Bacteriophage	ϕSA2 (*luk$_{PV}$SF*)
Pathogenicity island	SaPI3 (*seb1,sek,seq*)
Mobile element structure	MES$_{PM1}$ (*ermB, aph(3')-IIIa, aadE, cat)*, MES$_{tet}$ (*tet(K)*)
Transposon	Tn*553* (*blaZ-blaR1-blaI* operon), Tn*4001* (*aacA-aphD*)

* Gene for the formyl peptide receptor-like 1 inhibitor (FLIPr) involved in immune evasion.

† *agr1a,* a variant of *agr*1

Virulence factor genes are put in parentheses after the name of genomic islands, bacteriophage, and pathogenicity islands. Antibiotic resistance genes/operons are put in parentheses after the name of mobile element structures and transposons.

In Taiwan, the dominant CA-MRSA clone is clonal complex (CC) 59 MRSA composed of two genotypes: the Asian-Pacific clone, characterized as ST59/SCC*mec* IV/*pvl*-negative MRSA; and the Taiwan clone, characterized as ST59/SCC*mec* Vb (5C2&5)/*pvl*-positive MRSA (Chen, Huang, 2014). The Taiwan clone was shown to be more virulent with greater potential of causing severe infections than the Asia-specific clone (Chen *et al.*, 2013). Moreover, the Taiwan clone can additionally possess several distinctive features contributing to its prevalence and distribution across numerous Asia and European nations (Hung *et al.*, 2012).

Genetic structure of genomic islands and transposons carrying antibiotic-resistant genes

Analysis of the draft genome of strain BM85 identified genomic islands including vSAα, vSAβ and vSAγ, staphylococcal pathogenicity island SaPI3 and bacteriophage ϕSA2. Hence, the MRSA strain BM85 is certainly a typical strain of the Taiwan clone (Higuchi et al., 2010; Hung et al., 2012; Nguyen et al., 2020). Additionally, MRSA strain BM85 also possessed a unique mobile element structure MES$_{PM1}$ with a composite transposon of 21,832 bp in size that harbored *ermB, aph3'-IIIa, aadE,* and *cat* genes

conferring resistance to erythromycin, kanamycin, streptomycin, and chloramphenicol, respectively (Figure 2). In addition, the truncation and deletion of type I restriction-modification (R-M) system genes (*hsdS* and *hsdM*, respectively) in genomic island vSAβ was caused by the insertion of the immune evasion cluster (IEC) type C carrying two virulence genes *scn* and *chp* that protect *S. aureus* from innate immune systems (Hung et al., 2012). Notably, the type I restriction-modification system, consisting of *hsdR* restriction gene, *hsdM* modification gene, and *hsdS* sequence specificity gene, was shown to block the transfer of resistance genes and other mobile genetic elements from other species into *S. aureus*, as well as between different *S. aureus* lineages (Waldron & Lindsay, 2006). Therefore, deficiency in the type I R-M system would enhance genome plasticity by facilitating the acquisition of foreign genetic structures, such as MES_{PM1}.

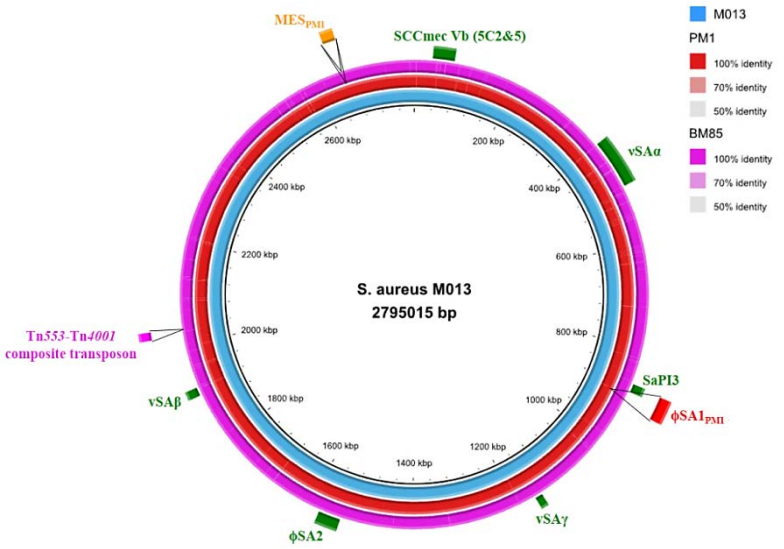

Figure 1. Comparative genomic analysis of MRSA strain BM85 and strain PM1 with the reference MRSA strain M013. The chromosomal draft genomes of two strains BM85 and PM1 were mapped to the complete genome of strain M013. The innermost circle (blue) shows the genome of M013, followed by PM1 (red) and eventually, MRSA strain BM85 (pink). Green, red, pink and orange arcs indicate the genetic elements (genomic islands, mobile elements, and bacteriophages) that were present at particular chromosomal locations in all three strains, only in PM1, only in MRSA strain BM85, and in both strains PM1 and BM85, respectively.

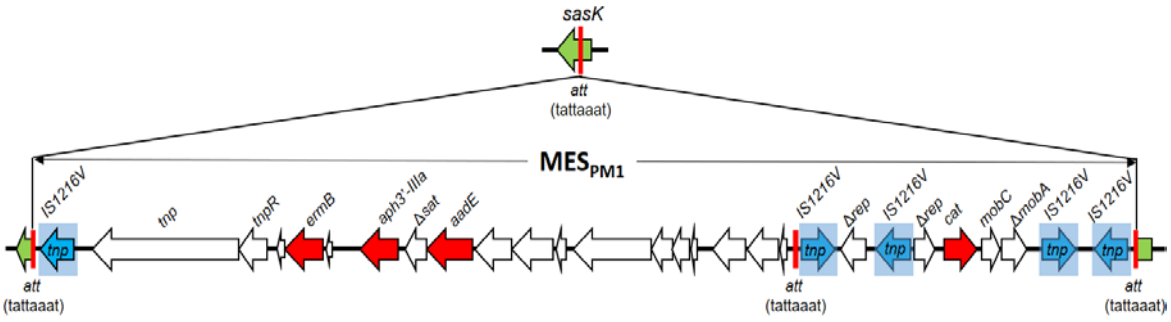

Figure 2. Genetic structure of MES_{PM1} carrying multiple antibiotic-resistant genes. The structure was derived from the study of Hung *et al*. 2012. Arrows represent ORFs and their transcriptional orientation. Red, blue, green arrows indicate antibiotic resistance genes, transposase genes of IS1216V, and *sasK* gene disrupted by MES_{PM1}, respectively. Attachment sites (*att*) with 8-bp direct repeats are indicated by red verticle lines. The region of insertion sequence IS*1216V* is shaded in blue. Greek delta symbol (Δ) indicates a truncated coding sequence.

Furthermore, a unique transposon structure of 13,615 bp formed by the insertion of transposon Tn*4001* into transposon Tn*553* was found in the chromosome of MRSA strain BM85 (Figure 3). This structure was designated as Tn*553*-Tn*4001* transposon and its structure is demonstrated in Figure 3. Tn*553* carried a complete *blaZ-blaR1-blaI* β-lactamase operon and was inserted into a specific site within the *yolD* gene in the BM85 chromosome with a 6-bp sequence of the left junction (5'-CAAAAG-3') different from that of the right junction (5'-TAAACG-3'). Within Tn*553*, a full sequence of Tn*4001* was integrated into an open reading frame encoding a hypothetical protein with 8-bp direct repeats (5'-AAATTTGT-3') produced at the attachment site. Tn*4001* was featured by two inverted repeats of IS256 flanking *aacA-aphD* gene which encodes aminoglycoside N(6')-acetyltransferase/aminoglycoside 2"-phosphotransferase, a bifunctional enzyme conferring resistance to gentamicin, tobramycin, and kanamycin.

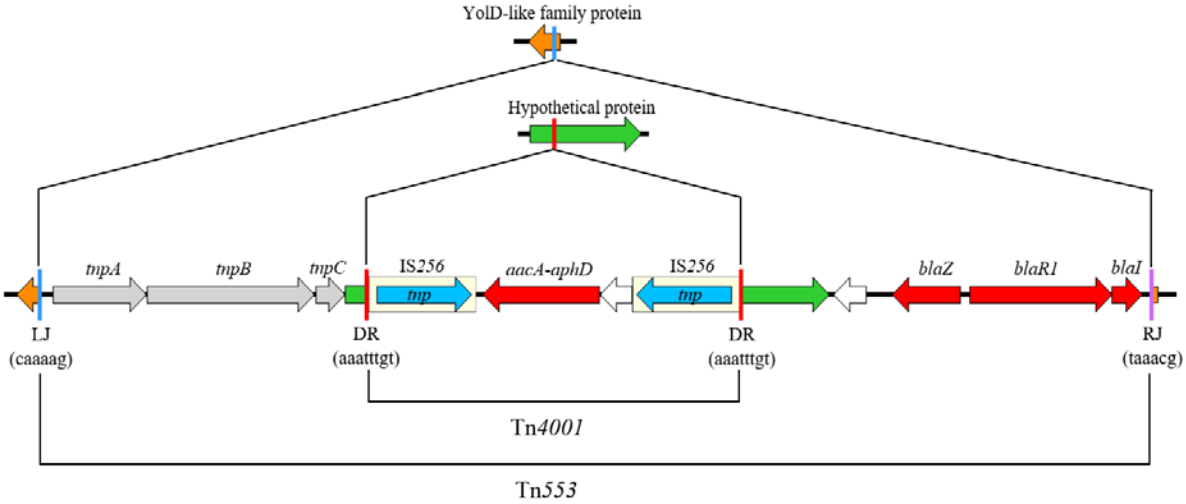

Figure 3. Genetic structure of the Tn*553*-Tn*4001* transposon in MRSA strain BM85. Arrows represent ORFs and their transcriptional orientation. Red, grey, blue, orange, green arrows show antimicrobial resistance genes, Tn*553* transposase genes *(tnpA*, *tnpB*, *tnpC),* Tn*4001* transposase gene *(tnp),* chromosomal ORF disrupted by Tn*553,* and ORF of Tn*553* disrupted by Tn*4001*, respectively. The left junction (LJ) representing the Tn*553* integration site and the right junction (RJ) formed upon Tn*553* transposition is indicated by blue vertical lines and purple vertical lines, respectively. The 8-bp direct repeats generated by duplication of target sequence on insertion of Tn*4001* into Tn*553* are indicated by red vertical lines. The region of insertion sequence IS*256* is shaded in yellow.

In addition, MRSA strain BM85 possessed a full-sequence plasmid pM013 harboring *tetK* gene responsible for tetracycline resistance, transposon MES_{tet} and cadmium resistance *cadD-cadX* operon. Plasmid pM013 was first identified and described in another Taiwan clone, MRSA strain M013 (Huang et al., 2012) which lacked MES_{PM1} and Tn*553*-Tn*4001* transposon; suggesting two possibilities: (i) the presence of pM013 in MRSA strain BM85 was due to a horizontal gene transfer (HGT) in the past between the progenitor of MRSA strain BM85 (carrying no plasmid) and strain M013 or (ii) strain M013 became the ancestor of MRSA strain BM85 after acquiring additional mobile genetic elements (MES_{PM1} and Tn*553*-Tn*4001* transposon) from other bacteria of Taiwan clone or different lineages via HGT overtime and eventually evolved into MRSA strain BM85. Collectively, the genome plasticity enhancement mediated by the intrinsic deficiency of type I R-M systems promoted HGT leading to the acquisition of various advantageous genetic structures from ambients, which subsequently contributed greatly to the increased antimicrobial resistance, virulence, adaptation, and dissemination of the Taiwan clone in general and MRSA strain BM85 in particular.

Correlation between antibiotic-resistant phenotype and genotype of MRSA strain BM85

A total of 21 complete AMR genes offering various mechanisms of drug resistance were identified in MRSA strain BM85 (Table 3). Out of 20 AMR genes, 13 of these (12 chromosomal genes and 1 plasmid-mediated gene) encode efflux pumps involved in the extrusion of a wide range of antimicrobials, and most of them belong to the major facilitator superfamily (MFS). Several efflux pumps are multidrug efflux pumps capable of extruding multiple structurally unrelated antimicrobial compounds such as antibiotic agents from different antibiotic classes, disinfectants, detergents, or dyes; therefore potentially promoting the expansion of antimicrobial resistance. Especially, all the genes encoding resistance-conferring enzymes come from mobile genetic elements described previously.

Table 3. Antibiotic susceptibility phenotype and antibiotic-resistant gene profiles of MRSA strain BM85.

Antibiotics	Phenotype	Respective resistance genes	
		Chromosome	Plasmid
Tetracycline	R	*tet38, mepA, norB*	*tetK*
Penicillin	R	*blaZ, mecA*	–
Methicillin	R	*mecA*	–
Cefoxitin	R	*mecA*	–
Gentamicin	R	*aph3'-IIIa, aacA-aphD*	–
Kanamycin	R	*aph3'-IIIa, aacA-aphD, lmrS*	–
Tobramicin	R	*aacA-aphD*	–
Erythromycin	R	*lmrS, ermB*	–
Ofloxacin	S	*norA, norB, norC*	–
Pristinamycin (Pristinammycin IA/IIA)	R	*ermB* (Pristinamycin IA)	–
Trimethoprim/Sulfamethoxazole	S	*lmrS*	–
Fusidic acid	S	*lmrS, mdeA*	–
Rifampin	S	*lmrS*	–
Fosfomycin	S	–	–

The antibiotic agents in the bracket indicate the substrate to which the gene confers resistance.

Abbreviation: R, resistant; I, intermediate; S, susceptible.

MRSA strain BM85 was found to be resistant to penicillin, cefoxitin, gentamicin, kanamycin, tobramycin, erythromycin, and tetracycline. This result is concordant with the resistant-genotypic profile since the MRSA strain BM85 carrying genes *tet38, tetK, blaZ, mecA, aph3'-IIIa, aacA-aphD,* and *ermB* are responsible for the resistances (Table 3). NorA, NorB, and NorC are multidrug efflux pumps responsible for quinolones resistance, however, the presence of genes encoding these proteins did not correlate with the resistance to ofloxacin. Similarly, LmrS and MdeA efflux pumps are capable of extruding a wide range of structurally unrelated

antimicrobials, the contribution of LmrS and MdeA to antibiotic resistance in BM85 was insignificant. In fact, chromosomally-encoded efflux pumps in Gram-positive bacteria often confer only a low level of resistance and the MDR occurs only when genes encoding those proteins are overexpressed due to mutations in regulatory genes/sequences (Schindler & Kaatz, 2016). These highlighted the significant contributions of mobile genetic elements to the MDR phenotype in MRSA strain BM85. Thus, the presence of multiple resistance cassettes in MDR *S. aureus* strains can be attributed to the high levels of non-prescription antibiotic usage seen in many Asian countries which had created selective pressures favoring the acquisition and stable maintenance of these structures in the genome.

CONCLUSION

In this study, the whole genome sequence analysis revealed that multidrug-resistant MRSA strain BM85 isolated from a Vietnamese patient with bloodstream infection was found to belong to the Taiwan clone of the genotype ST59/SCC*mec*Vb (5C2&5)/*pvl*-positive. The MRSA strain BM85 possessed a tetracycline resistance-encoding plasmid, numerous antibiotic resistance-conferring mobile genetic elements and pathogenicity islands. Overall, the majority of MDR phenotypes of MRSA strain BM85 were mediated by AMR genes in mobile genetic elements. Our data reinforce the acquisition of foreign resistance cassettes which was the main mechanism contributing largely to the MDR phenotypes of MRSA strain BM85.

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