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Correlation Analysis between Antibiotic Resistance Gene Profile and Susceptibility to Gentamicin, Clindamycin, and Minocycline in Clinically Isolated Methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

This study aimed to elucidate retrospectively the correlations between the genome and phenotype in clinical methicillin-resistant *Staphylococcus aureus* (MRSA) gentamicin (GEN), clindamycin (CLI), and minocycline (MIN) susceptibility using next-generation sequencing (NGS) technology. Ninety two MRSA strains were isolated from individual inpatients treated in Hiroshima University Hospital, Hiroshima, Japan, extracted for their genomic DNA, and sequenced using an Illumina® MiSeq sequencer to obtain their *de novo* whole-genome assembly. An *in silico* analysis using ResFinder was performed to obtain the genomic antimicrobial susceptibility profile which was analyzed together with GEN, CLI, and MIN minimum inhibitory concentration (MIC) levels. This study found *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ MRSA strains were predominant (42/92) and were shown to exhibit >16 mg/L GEN (40/42), >4 mg/L CLI (26/42), and >8 mg/L MIN MIC levels (30/42). Associations between *aac(6')aph(2")* detections and GEN MIC levels ($p < 0.001$), *ermA* detections and CLI MIC levels ($p < 0.001$), and *tetM* detections and MIN MIC levels ($p < 0.001$) were revealed in this study. Correlations between simultaneous detections of *aac(6') aph(2")-spc-ermA-tetM* and GEN MIC levels ($\varphi_c = 0.398$, $p < 0.001$), CLI MIC levels ($\varphi_c = 0.448$, $p < 0.001$), and MIN MIC levels ($\varphi_c = 0.515$, $p < 0.001$) were revealed in this study. The genomic-phenotypic correlation analyses in this study provided an insight of a rapid antimicrobial detection in MRSA using *in silico* genomic antimicrobial susceptibility profiling.

Key words: *Methicillin-resistant Staphylococcus aureus, next-generation sequencing, in silico genome analysis, antimicrobial susceptibility*

Rapid antimicrobial susceptibility surveillance as a part of the clinical methicillin-resistant *Staphylococcus aureus* (MRSA) infection control is required as previous reports have shown the rise in MRSA-related mortalities and the increase of multi-drug non-susceptible strains[8,15]. The use of next-generation sequencing (NGS) technology would provide less laborious efforts, compared to the conventional method, for rapid antimicrobial susceptibility detection in clinical isolates by *in silico* susceptibility-related genes identification[17].

Previous studies reported the use of DNA-based antimicrobial susceptibility genome profiling in clinical MRSA outbreaks[1,10]. These findings would suggest that NGS is a promising method for rapid antimicrobial susceptibility detection in MRSA infection control. The benefit of *in silico* genome detection of antimicrobial susceptibility would be in

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the greater effect to the commonly administered antimicrobial in MRSA infections, such as gentamicin (GEN), clindamycin (CLI), and minocycline (MIN)[12].

This study was designed to reveal the phenotype of antimicrobial susceptibility from the observation of MIC level, to reveal the genomic antimicrobial susceptibility profile identified through *in silico* analyses of draft *de novo* assembly, and to reveal the correlations between genome and phenotype of antimicrobial susceptibility of clinical MRSA strains as observed in GEN, CLI, and MIN.

METHODS

Isolation and Antimicrobial Susceptibility of MRSA Strains

Ninety two bacterial strains were isolated from the specimens of individual inpatients treated at Hiroshima University Hospital, Hiroshima, Japan during two separate time periods: October – December 2009 (36/92) and January 2013 – March 2014 (56/92). Isolates were preserved in a heart infusion broth-10% glycerol mixture and stored at -80°C. Antimicrobial susceptibility was measured with an image analyzer (Koden IA40MIC-i, Koden, Tokyo, Japan) using a dry plate (Eiken, Tokyo, Japan). The procedure examined and reported the cefoxitin, GEN, CLI, and MIN MIC level according to the modified standards of the CLSI M100-S19 guideline, which was used in Hiroshima University Hospital, Hiroshima, Japan as a part of routine clinical laboratory examination. All *S. aureus* isolates in this study exhibited a cefoxitin MIC of >4 mg/L and were classified as MRSA (data not shown). The GEN, CLI, and MIN MIC data were categorized according to the guideline described above during the procedure. A GEN non-susceptible strain was defined as a strain which exhibited ≥8 mg/L GEN MIC level. A CLI non-susceptible strain was defined as a strain which exhibited ≥2 mg/L CLI MIC level. A MIN non-susceptible strain was defined as a strain which exhibited >8 mg/L MIN MIC level.

Extraction and Sequencing of MRSA Genomic DNA

A glycerol stock of each isolate was used to inoculate heart infusion agar plates (E-MC85 Pearlcore®; Eiken, Tokyo, Japan), which were incubated for 12 hr at 37°C. A single colony from the plate was removed and cultured in blood heart infusion liquid medium (E-MC04; Eiken, Tokyo, Japan) using a water bath for 12 hr at 37°C. The culture was then centrifuged at 1600 × *g* for 10 min at room temperature to obtain a pellet. Genomic DNA was extracted from the pellet using a QIAamp® DNA Mini kit (Qiagen, Venlo, the Netherlands) and sequenced to obtain paired-end reads using a Nextera® XT DNA kit (Illumina, San Diego, CA) and an Illumina® MiSeq sequencer (Illumina, San Diego, CA).

Draft *de novo* Genome Assembly of MRSA Strains

The draft *de novo* genome assembly was assembled from the paired-end reads using SPAdes v3.5.0 by the GABenchToB recommendations and was reoriented into the *S. aureus* N315 chromosomal sequence (RefSeq GCA_000009645.1) using Mauve v2.4.0 [2,9,11,14].

In silico Genomic Antimicrobial Susceptibility Profile of MRSA Strains

The draft assembly sequence was aligned using a ResFinder v2.1 online gene-mapping tool at >90% identity similarity and >60% query length thresholds[18]. This procedure aligned an assembly sequence into the database of acquired antimicrobial susceptibility genes stored in ResFinder and reported a panel of those gene matches by its antimicrobial class. A match of an aligned assembly sequence to a gene at the thresholds described above was reported as a positive finding. This procedure allowed the identification of two genes related in their acquired aminoglycoside susceptibility, which were *aac(6')aph(2")* and *spc*, a gene related in the acquired macrolide-lincosamide-streptogramin B susceptibility, which was *ermA*, and a gene related in the acquired tetracycline susceptibility, which was *tetM,* from MRSA strains in this study. The combined gene detection pattern was then constructed from these genes.

Statistical Analysis

Statistical data analysis was performed using R v.3.3.1 aided by the R Commander v.2.2-5[6,13]. All statistical analyses were performed at a significant value of $p < 0.05$. The χ^2 test was performed to describe the associations between individual *in silico* detection of acquired antimicrobial susceptibility genes and GEN, CLI, and MIN MIC levels at its respective non-susceptible threshold. The Fisher-Freeman-Halton exact test of independence was performed and the Cramer's V coefficient was calculated to describe the correlations between the genomic detection patterns and the MIC levels of MRSA strains as observed in GEN, CLI, and MIN.

RESULTS

Minimum Inhibitory Concentration Levels of GEN, CLI, and MIN in MRSA Strains

Non-susceptibility to GEN was found in 75.00% (69/92) strains. Most of these strains (91.30%, 63/69) were shown to exhibit >16 mg/L GEN MIC level. Among the GEN-susceptible strains (25%, 23/92), most strains (82.61%, 19/23) were shown to exhibit ≤0.5 mg/L GEN MIC level. Non-susceptibility to CLI was found in 68.48% (63/92) strains. Most of these strains (80.95%, 51/63) were shown

to exhibit >4 mg/L CLI MIC level. Among the CLI-susceptible strains (31.52%, 29/92), most strains (93.10%, 27/29) were shown to exhibit ≤0.12 mg/L CLI MIC level. Non-susceptibility to MIN was found in 40.22% (37/92) strains and all of these strains were shown to exhibit >8 mg/L MIN MIC level. Among the MIN-susceptible strains (59.78%, 55/92), most strains (50.90%, 28/55) were shown to exhibit ≤1 mg/L MIN MIC level.

Detection Patterns of *aac(6')aph(2")*, *spc*, *ermA*, and *tetM*in MRSA Strains

The MRSA *in silico* acquired antimicrobial susceptibility detection revealed 63.04% (58/92) strains were *aac(6')aph(2")*$^+$, 77.17% (71/92) strains were *spc*$^+$, 77.17% (71/92) strains were *ermA*$^+$, and 69.57% (64/92) strains were *tetM*$^+$. All of the *spc*$^+$ strains were *ermA*$^+$ strains. From these results, six gene detection patterns were identified and consisted of *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ as seen in 45.65% (42/92) strains; *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^-$ as seen in 1.09% (1/92) strains; *aac(6')aph(2")*$^-$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ as seen in 23.91% (22/92) strains; *aac(6')aph(2")*$^-$, *spc*$^+$, *ermA*$^+$, *tetM*$^-$ as seen in 6.52% (6/92) strains; *aac(6')aph(2")*$^+$, *spc*$^-$, *ermA*$^-$, *tetM*$^-$ as seen in 16.30% (15/92); and *aac(6')aph(2")*$^-$, *spc*$^-$, *ermA*$^-$, *tetM*$^-$ as seen in 6.52% (6/92) strains. The *aac(6')aph(2")*$^+$, *spc*$^-$, *ermA*$^-$, *tetM*$^+$ and *aac(6')aph(2")*$^-$, *spc*$^-$, *ermA*$^-$, *tetM*$^+$ patterns were not found.

Individual Detection of *aac(6')aph(2")*, *spc*, *ermA*, and *tetM* and Minimum Inhibitory Concentration Level of GEN, CLI, and MIN in MRSA Strains

Positive finding of *aac(6')aph(2")* was associated with the GEN MIC level at its non-susceptible threshold (Table 1), while positive findings of *spc*, *ermA,* and *tetM* were associated with the CLI (Table 2) and MIN (Table 3) MIC levels at its non-susceptible threshold. Most *aac(6')aph(2")*$^+$ strains were shown to exhibit non-susceptible GEN MIC levels. The *aac(6')aph(2")*$^-$ strains were shown to exhibit either susceptible or non-susceptible GEN MIC levels. Most *spc*$^+$ strains were shown to exhibit non-susceptible CLI and MIN MIC levels while most *spc*$^-$ strains were shown to exhibit susceptible CLI and MIN MIC levels. Similar findings of *spc* in CLI and MIN susceptibility were shown during the detection of *ermA* and *tetM*.

Minimum Inhibitory Concentration Level and Detection Pattern in GEN, CLI, and MIN Susceptibility in MRSA Strains

The GEN MIC level was shown to be in a moderate correlation with the detection patterns and the involvement of *aac(6')aph(2")* was emphasized

Table 1. Detection of *aac(6')aph(2")* revealed to be associated with the GEN MIC levels at non-susceptible threshold

Gene Detections	GEN MIC Level (mg/L)						p of S v NS
	S			NS			
	≤0.5	1	4	8	16	>16	
aac(6')aph(2") +/-	3/16	0/3	1/0	2/0	4/0	48/15	<0.001
spc +/-	15/4	3/0	0/1	0/2	0/4	53/10	0.886
ermA +/-	15/4	3/0	0/1	0/2	0/4	53/10	0.886
tetM+/-	11/8	2/1	0/1	0/2	0/4	51/12	0.116

Abbreviations: GEN, gentamicin; MIC, minimum inhibitory concentration; S, susceptible; NS, non-susceptible

Table 2. Detection of *spc*, *ermA*, and *tetM* revealed to be associated with the CLI MIC levels at non-susceptible threshold

Gene Detections	CLI MIC Level (mg/L)						p of S v NS
	S				NS		
	≤0.12	0.25	0.5	1	2	>4	
aac(6')aph(2") +/-	20/7	0/1	1/0	0/0	11/1	26/25	0.206
spc +/-	10/17	1/0	1/0	0/0	12/0	47/4	<0.001
ermA +/-	10/17	1/0	1/0	0/0	12/0	47/4	<0.001
tetM+/-	8/19	0/1	1/0	0/0	11/1	44/7	<0.001

Abbreviations: CLI, clindamycin; MIC, minimum inhibitory concentration; S, susceptible; NS, non-susceptible

Table 3. Detection of *spc*, *ermA*, and *tetM* revealed to be associated with MIN MIC levels at non-susceptible threshold

Gene Detections	MIN MIC Level (mg/L)					*P* of S v NS
	S				NS	
	≤1	2	4	8	>8	
aac(6')aph(2") +/-	19/9	3/3	3/5	3/10	30/7	0.522
spc +/-	10/18	6/0	8/0	12/1	35/2	<0.001
ermA +/-	10/18	6/0	8/0	12/1	35/2	<0.001
tetM+/-	3/25	6/0	8/0	12/1	35/2	<0.001

Abbreviations: MIN, minocycline; MIC, minimum inhibitory concentration; S, susceptible; NS, non-susceptible

(φ_c= 0.398, p <0.001) (Table 4). The analysis revealed strains whose detection pattern at least consisted of *spc*$^+$*ermA*$^+$ and one additional gene was shown to exhibit >16 mg/L GEN MIC level. The effect was found to be greater in the addition of both *aac(6')aph(2")* and *tetM* as seen in *aac(6')aph(2")*$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$ strains. Singular detection of *aac(6')aph(2")* itself was found in strains exhibiting >16 mg/L GEN MIC level. The CLI MIC level was shown to be in a moderate correlation with the detection patterns and the involvements of *spc* and *ermA* were emphasized (φ_c= 0.448, p <0.001) (Table 5). The analysis revealed strains whose detection pattern at least consisted of *spc*$^+$*ermA*$^+$ and one additional gene shown to exhibit >4 mg/L CLI MIC level. The effect was found to be greater in the addition of *tetM*, as seen in *aac(6')aph(2")*$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$, and was found to be greatest in the addition of both *aac(6')aph(2")* and *tetM* as seen in *aac(6')aph(2")*$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$ strains. Singular detection of *aac(6')aph(2")* itself was found in strains exhibiting ≤0.12mg/L CLI MIC level. The MIN MIC level was shown to be in a strong correlation with the detection patterns and the involvement of *tetM* was emphasized (φ_c= 0.515, p <0.001) (Table 6). Strains whose pattern included *tetM*, as seen in *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ and *aac(6')aph(2")*$^-$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ strains, were shown to exhibit >8 mg/L MIN MIC level. Strains whose pattern included only *spc-ermA*, as seen in *aac(6')aph(2")*$^-$, *spc*$^+$, *ermA*$^+$, *tetM*$^-$ strains, and included only *aac(6')aph(2")*, as seen in *aac(6')aph(2")*$^+$, *spc*$^-$, *ermA*$^-$, *tetM*$^-$ strains, were shown to exhibit ≤1mg/L MIN MIC levels.

Trends in Detection Patterns and GEN, CLI, and MIN Susceptibility between MRSA Strains of Two Periods

Strains which possessed an *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ pattern were revealed to be in increasing trends between 2009 and 2013 – 2014 and most were shown to exhibit >16 mg/L GEN MIC level (Fig. 1), >4 mg/L CLI MIC level (Fig. 2), and >8 MIN MIC level (Fig. 3). The *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^-$ pattern was found only in a 2013 – 2014 strain and was shown to exhibit >16 mg/L

Table 4. Genomic detection patterns revealed to be correlated with GEN MIC levels.

Detection Patterns	GEN MIC Level (mg/L)						Total	φ_c	p
	≤0.5	1	4	8	16	>16			
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	2	0	0	0	0	40	42		
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	0	0	0	0	0	1	1		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	9	2	0	0	0	11	22		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	4	1	0	0	0	1	6	0.398	<0.001
aac(6')aph(2")$^+$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	1	0	1	2	4	7	15		
aac(6')aph(2")$^-$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	3	0	0	0	0	3	6		
Total	**19**	**3**	**1**	**2**	**4**	**63**	**92**		

Abbreviations: CLI, clindamycin; MIC, minimum inhibitory concentration

Table 5. Genomic detection patterns revealed to be correlated with CLI MIC levels.

Detection Patterns	CLI MIC Level (mg/L)						Total	φ_c	p
	≤0.12	0.25	0.5	1	2	>4			
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	5	0	1	0	10	26	42		
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	0	0	0	0	1	0	1		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	3	0	0	0	1	18	22		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	2	1	0	0	0	3	6	0.448	<0.001
aac(6')aph(2")$^+$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	15	0	0	0	0	0	15		
aac(6')aph(2")$^-$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	2	0	0	0	0	4	6		
Total	**27**	**1**	**1**	**0**	**12**	**51**	**92**		

Abbreviations: CLI, clindamycin; MIC, minimum inhibitory concentration

Table 6. Genomic detection patterns revealed to be correlated with MIN MIC levels.

Detection Patterns	MIN MIC Level (mg/L)					Total	φ_c	p
	≤1	2	4	8	>8			
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	3	3	3	3	30	42		
aac(6')aph(2")$^+$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	1	0	0	0	0	1		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^+$	0	3	5	9	5	22		
aac(6')aph(2")$^-$,*spc*$^+$,*ermA*$^+$,*tetM*$^-$	6	0	0	0	0	6	0.515	<0.001
aac(6')aph(2")$^+$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	15	0	0	0	0	15		
aac(6')aph(2")$^-$,*spc*$^-$,*ermA*$^-$,*tetM*$^-$	3	0	0	1	2	6		
Total	**28**	**6**	**8**	**13**	**37**	**92**		

Abbreviations: MIN, minocycline; MIC, minimum inhibitory concentration

GEN MIC level (Fig. 1), 2 mg/L CLI MIC level (Fig. 2), and ≤1 mg/L MIN MIC level (Fig. 3). The detection of *aac(6')aph(2")*$^-$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ strains between two periods was shown to be in decreasing trends and was accompanied by decreasing trends of GEN MIC levels (Fig. 1), CLI MIC levels (Fig. 2), and MIN MIC levels (Fig. 3) among those strains. The detection of *aac(6')aph(2")*$^-$,*spc*$^+$,*ermA*$^+$, *tetM*$^-$ strains between two periods was found to be in increasing trends and was accompanied by in-

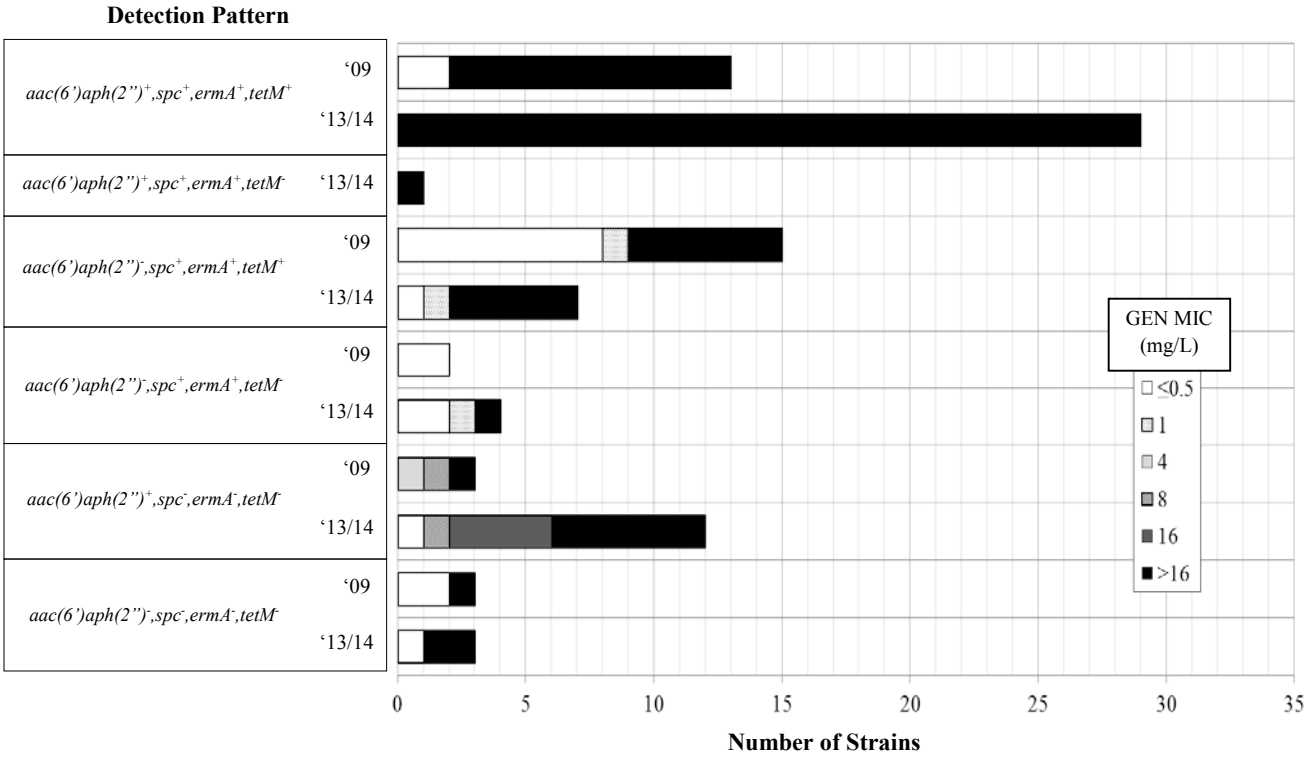

Fig. 1. Genomic detection patterns and GEN MIC level distributions between strains isolated from 2009 and 2013 - 2014

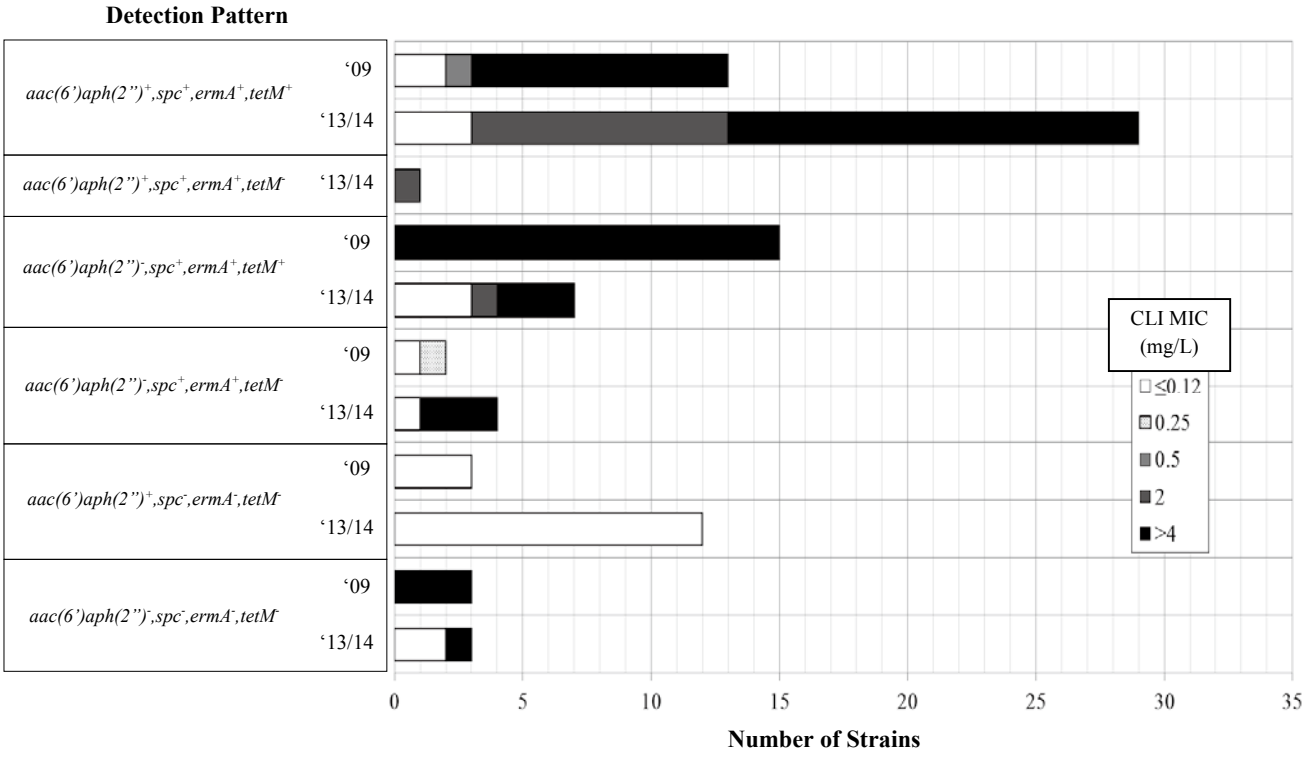

Fig. 2. Genomic detection patterns and CLI MIC level distributions between strains isolated from 2009 and 2013 - 2014

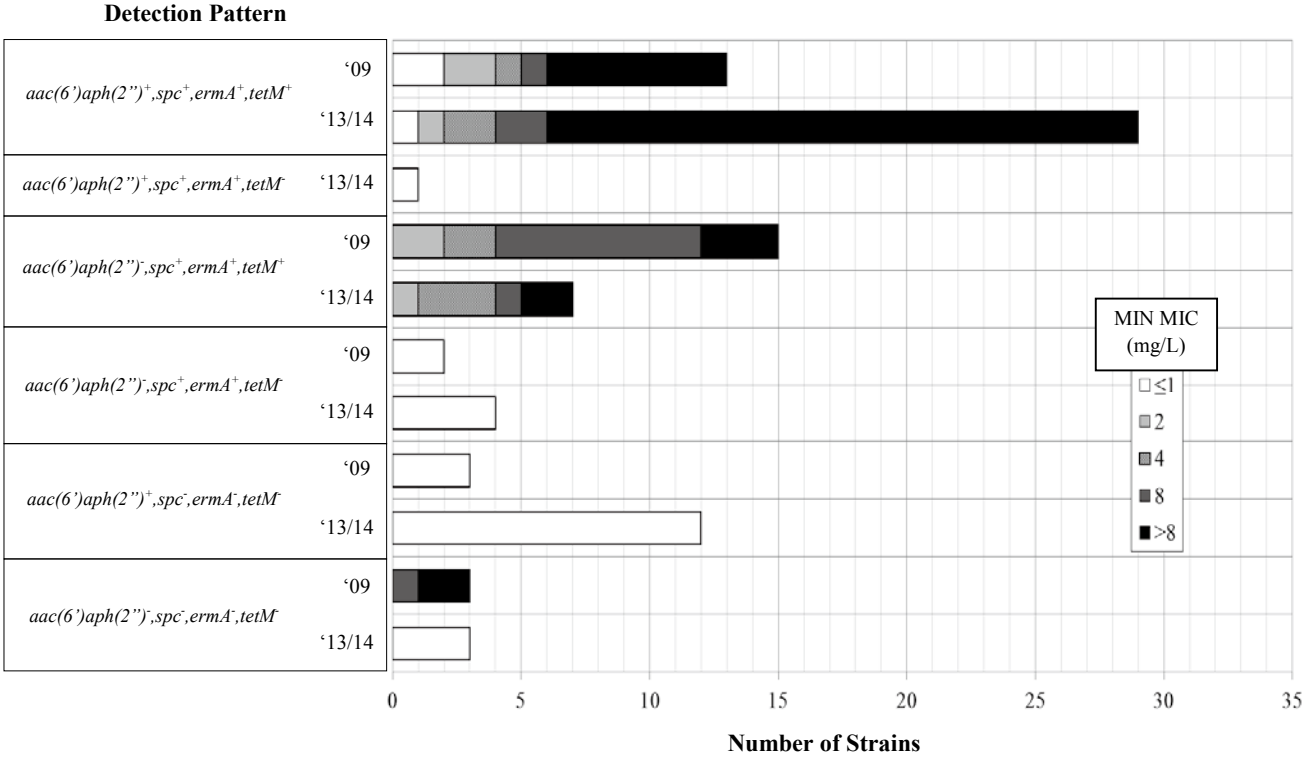

Fig. 3. Genomic detection patterns and MIN MIC level distributions between strains isolated from 2009 and 2013 - 2014

creasing trends of GEN MIC levels (Fig. 1) and CLI MIC levels (Fig. 2) among those strains. The MIN MIC levels of these strains were shown to be steady at ≤1 mg/L between two periods (Fig. 3). The *aac(6')aph(2")*$^+$, *spc*$^-$, *ermA*$^-$, *tetM*$^-$ strains which exhibited 16 mg/L and >16 mg/L GEN MIC level were found to be in increasing trends (Fig. 1). The CLI MIC levels (Fig. 2) and MIN MIC levels (Fig. 3) of these strains were shown to be steady at ≤0.12 mg/L and ≤1 mg/L, respectively, between two periods. The GEN MIC level of *aac(6')aph(2")*$^-$, *spc*$^-$, *ermA*$^-$, *tetM*$^-$ strains between two periods was found to be in increasing trends (Fig. 1), while CLI (Fig. 2) and MIN MIC levels (Fig. 3) of these strains were found to be in decreasing trends.

DISCUSSION

This study demonstrated the use of NGS technology techniques in obtaining the genomic antimicrobial susceptibility profile and showed the correlation of the genomic antimicrobial susceptibility profile to the MIC levels in MRSA strains. The genomic antimicrobial profiling performed in this study revealed the co-existence of acquired genes in GEN, CLI, and MIN susceptibility in most MRSA strains which exhibited high GEN, CLI, and MIN MIC levels, of which *aac(6')aph(2")*$^+$, *spc*$^+$, *ermA*$^+$, *tetM*$^+$ MRSA strains were shown to be in increasing trends. This important finding would suggest that the emergence of multidrug non-susceptibility strains among the clinical MRSA strains would warrant rapid antimicrobial susceptibility detection provided by the *in silico* combined detection of *aac(6')aph(2")-spc-ermA-tetM*.

The use of ResFinder as described in this study allowed investigation of multiple acquired antimicrobial susceptibility genes at the same time. The tool also allowed detection of point mutations and/or deletions along with the wild type sequence of each gene. This study found three *aac(6')aph(2")*$^+$ strains with 157C>T mutation and six *aac(6')aph(2")*$^+$ strains with deletion, and all of these strains exhibited more than 8 mg/L GEN MIC level. A single detection of *spc*$^+$ strain with 267G>C mutation was shown to exhibit >16 mg/L GEN MIC level, >4 mg/L CLI MIC level, and >8 mg/L MIN MIC level. Twelve *tetM*$^+$ strains with simultaneous 1040G>A and 1636A>G mutations were found to exhibit more than 4 mg/L MIN MIC level except in one strain which exhibited 2 mg/L MIN MIC level, suggesting *tetM* variations or polymorphisms. Two *ermA*$^+$ strains with 202T>C mutation exhibiting less than 0.5 mg/L CLI MIC levels were found, suggesting a mutation which could cause disruption in the gene function (data not shown).

A previous study used NGS technology techniques and reported the association between the detection of antimicrobial genes and MIC level in GEN and CLI susceptibility at a greater number of MRSA isolates. The study revealed an association between *ermA* and tetracycline MIC level, however, the association between GEN-related genes and GEN MIC level was not firmly estab-

lished[7] The present study addressed this problem and found that the detection of *aac(6')aph(2")* was correlated to the GEN MIC level. The number of *aac(6')aph(2")*$^+$ MRSA strains whose exhibiting >8 mg/L GEN MIC level found in this study (58.69%, 54/92) was slighty comparable to that of a previous study, which reported the existence of *aac(6') aph(2")* in 88.89% (8/9) strains exhibiting >8 mg/L GEN MIC level[16]. The present study revealed an association between *spc*, *ermA*, and *tetM* and the CLI MIC levels, however, a discrepancy occurred. The *spc* gene was associated with the spectinomycin susceptibility and it concurrently existed with *ermA* as observed in transposon Tn554 of food-borne MRSA CC398 strains[4]. This would suggest involvement of *ermA*, rather than *spc*, in CLI susceptibility. A previous study reported a cross non-susceptibility between erythromycin and CLI involving *ermA* in MRSA, however, without firm association with the CLI MIC level[3]. The study mentioned the importance of distinguishing erythromycin and CLI non-susceptibility during *ermA* detection in MRSA strains due to the nature of the *erm* class gene. The erythromycin susceptibility test was not a part of routine examination in the present study location which may limit the interpretation of the correlation analysis involving CLI MIC level. Future study involving erythromycin would be warranted to improve the accuracy of this part of the study result. Greater non-susceptibility of CLI in *ermA*$^+$ MRSA involving *aac(6') aph(2")* and *tetM* genes was of unknown mechanism and future study addressing these findings should be warranted. The MIN MIC level was shown to be correlated to the existence of *tetM* as supported by a previous study[5].

Several limitations existed during the *in silico* detection of antimicrobial susceptibility genes. The given statistical results of this study are subject to sample bias, wherein nosocomial MRSA strains could be originated from the same clone. The strains included in this study were obtained from unique patients from each era. In addition, although similar clinical features existed between the strains, our NGS data suggested these strains were of different clones. Genome assembly comparison analysis between each of the strains from the NGS data was performed and none of these genomes were 100.00% similar, although the genome similarity was found to be approximately 96.00 – 99.00% similar (data not shown). Non-detection of other MRSA antimicrobial susceptibility genes in this study could be attributed to the genomic characteristics of the clinically isolated Japanese MRSA clone itself which might be different from that of other clones from different regions. The discrepancy between the genomic and phenotypic profile could rise from unidentified novel gene variants which may in turn be responsible in different non-susceptibility mechanisms. Gene expression is a result of a complex mechanism which may also involve molecules remotely located from the detected gene itself. These conditions would limit the availability of the antimicrobial genes within the database. Discrepancy could also be reduced by single observations of MIC level between appropriate antimicrobial class and its respective susceptibility genes, instead of combined simultaneous detection of antimicrobial susceptibility genes. Combined use of NGS technology based techniques and other methods would effectively provide accurate and reliable information, as laboratory methods used to detect antimicrobial susceptibility in MRSA should have high sensitivity and specificity. Nevertheless, this study showed a promising method of *in silico* detection of antimicrobial susceptibility using NGS data in MRSA strains.

In conclusion, the *in silico* detection of *aac(6') aph(2")-spc-ermA-tetM* would provide an insight of a rapid antimicrobial susceptibility detection in MRSA infection control during the era of multidrug non-susceptible MRSA strains.

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