論　文　内　容　要　旨

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

（メチシリン耐性黄色ブドウ球菌の臨床分離株における抗菌薬耐性遺伝子分布とゲンタマイシン、クリンダマイシン、ミノサイクリン感受性の関連についての解析）

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Background

Rapid antimicrobial susceptibility surveillance is essential in the clinical methicillin-resistant *Staphylococcus aureus* (MRSA) infection control as the rise in MRSA-related mortalities and the increase of multi-drug non-susceptible strains have become apparent. The use of next generation sequencing (NGS) technology allows DNA-based genome profiling in clinical MRSA outbreaks and requires less laborious effort, compared to conventional methods, for the purpose of MRSA antimicrobial susceptibility surveillance. This study aimed to retrospectively elucidate the correlations between the genome and phenotype in clinical MRSA gentamicin (GEN), clindamycin (CLI), and minocycline (MIN) susceptibility using NGS technology. The benefit of antimicrobial susceptibility surveillance using NGS technology would be in the greater effect to these commonly administered antimicrobials in MRSA infections.

Methods

Ninety two MRSA strains were isolated from individual inpatients treated in Hiroshima University Hospital, Hiroshima, Japan during two separate time periods: October – December 2009 (36/92) and January 2013 – March 2014 (56/92). The minimum inhibitory concentration (MIC) data for GEN, CLI, and MIN (GEN-MIC, CLI-MIC, MIN-MIC) of the strains were categorized according to the modified standards of the CLSI M100-S19 guideline during the procedure. A GEN non-susceptible strain was defined as a strain which exhibited ≥8 mg/L of GEN-MIC level. A CLI non-susceptible strain was defined as a strain which exhibited ≥2 mg/L of CLI-MIC level. A MIN non-susceptible strain was defined as a strain which exhibited >8 mg/L MIN MIC level. Genomic DNAs were isolated from the strains and sequenced using an Illumina® MiSeq sequencer. The sequence data of each strain was assembled using SPAdes to obtain its *de novo* whole-genome assembly. An *in silico* analysis using ResFinder online gene-mapping tool was performed at >90% identity similarity and >60% query length thresholds to obtain the genomic antimicrobial susceptibility profile from each of the assembly. This procedure allowed the identification of two genes related in the 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.* The resulting profile was analyzed together with GEN, CLI, and MIN-MIC levels. 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 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 & Discussion

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 of 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 of 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 of MIN-MIC level. The *in silico* 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 *spc*$^{+}$ strains were *ermA*$^{+}$ strains. 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. Six gene detection patterns were identified and were 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. The simultaneous detection patterns were correlated with GEN-MIC levels ($\varphi_c = 0.398$, $p < 0.001$), CLI-MIC levels ($\varphi_c = 0.448$, $p < 0.001$), and MIN-MIC levels ($\varphi_c = 0.515$, $p < 0.001$). The predominating *aac(6')aph(2")*$^{+}$,*spc*$^{+}$,*ermA*$^{+}$,*tetM*$^{+}$ MRSA strains 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) and its presence was shown to be in increasing trends between 2009 and 2013 – 2014. The genomic-phenotypic correlations analyses in this study provided an insight of a rapid antimicrobial detection in MRSA using *in silico* genomic antimicrobial susceptibility profiling. The correlation between the detection of *aac(6')aph(2")* and GEN-MIC level revealed in this study were comparable to that of a previous study. The study mentioned the importance to distinguish erythromycin and CLI non-susceptibility during *ermA* detection in MRSA strains due to the nature of *erm* class gene. Since the erythromycin susceptibility test was not examined in the routine examination in our hospital, it may limit to interpret the correlation analysis involving CLI-MIC level. Future study involving erythromycin would be warranted to improve the accuracy of this part of 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. This study suggest the detection of the emerging multidrug non-susceptibility strains among the clinical MRSA strains could be performed using *in silico* combined detection of *aac(6')aph(2")-spc-ermA-tetM*. Discrepancy occurred in this study could be attributed to the unidentified novel gene variants which may in turn were responsible in different non-susceptibility mechanisms. Combined use of NGS technology and other methodologies would effectively provide accurate and reliable information, as laboratory methods used to detect antimicrobial susceptibility and mechanism in MRSA with high sensitivity and specificity.