ABSTRACT OF THE THESIS

Molecular characterization of *Staphylococcus aureus* isolated from skin infection

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Molecular epidemiological characterization of total 540 isolates of *Staphylococcus aureus* from skin infection in outpatient hospitals and private clinics collected in a clinical laboratory center in West Japan from 2015 to 2018 was conducted. The isolates included 315 from pus discharge of open wound, 108 from pus discharge of closed abscess, and 117 from pressure ulcers. Major clonal complexes (CCs) of these isolates were CC8 (20.9%), CC121 (13.5%), CC15 (13.1%), and CC5 (11.7%). Each CC possessed a specific coagulase serotype and SCC*mec* type. Overall prevalence of *mecA* was 40.2% but the prevalence was significantly different among isolates of three origins: the percentage of *mecA* of isolates from open wound, closed abscess, and pressure ulcer were 38.1%, 13.9% and 70.1% respectively. Major CCs of isolates from each infection origin were different: those from open wound were CC121, CC8, CC15, closed abscess were CC15, CC8, CC45, and pressure ulcer were CC8, CC5, CC1 respectively. Prevalence of *mecA* in each CC was strikingly

different, and each CC possessed a unique repertoire of virulence and antimicrobial resistance genes. CC121, which is more common in isolates from open wound, had a high positive rate of exfoliative toxin A gene, suggesting that exfoliative toxin is a pathogenic factor associated with open wound. CC5 carried SCC*mec* type II and showed much more antimicrobial resistance than other CCs. Most of the isolates from pressure ulcer were MRSA displaying multiple antimicrobial resistance against gentamicin, macrolide, and new quinolone. CC8 was the common lineage present in the isolates of three types of skin infections and ST8 CA-MRSA/J and CA-MSSA/J, carrying multiple virulence genes, represented 17.7 and 15.0% respectively whereas *pvl*-positive USA300 lineage (ST8 SCC*mec* type IVa) was only 1.8% of CC8. Most of these lineages were isolated from open wound or closed abscess. On the other hand, most of ST8 isolates from pressure ulcer carried SCC*mec* type I and multiple resistance genes but few virulence genes. These results illustrated unique characteristics of major strain lineages causing skin infections and strongly suggested that there is a strong association between genotype and pathogenic potential of *S. aureus* causing different types of skin infection.

Among the skin isolates, we found 11 isolates were oxacillin-susceptible but *mecA*-positive showing characteristics of OS-MRSA. Those isolates were determined as MSSA by an automated antimicrobial susceptibility testing but were positive for *mecA* by PCR. Whole genome sequencing indicated all 11 isolates belonged to ST121 and carried SCC*mec* type V. All these isolates possessed the same one base substitution in the promoter sequence of *mecA* in common: the 18th base in the

MecI/BlaI-binding palindrome structure of the *mecA* repressor was substituted from C to T. This C to T substitution was reported to be involved in attenuating *mecA* transcription and corresponding PBP2′ (PBP2A) production, and was found to be a common nucleotide substitution in the *S. aureus* carrying SCC*mec* type V lineages. In addition, all of these strains increased the oxacillin MIC in the presence of mupirocin (0.03 μg/ml) which has been reported to increase *mecA* transcription and PBP2′ production through inducing stringent responses. Most isolates of ST121 are producing exfoliative toxin A and major causative agent of bullous impetigo in children. This finding calls attention not to make a medication mistake in case of treating patient with staphylococcal bullous impetigo since β-lactam antibiotics are the first-line drugs in case the causative agent is diagnosed as methicillin-susceptible *S. aureus*.