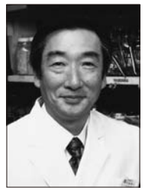

Methicillin-resistant *Staphylococcus aureus*: a comprehensive view

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I. MRSA: Reversal of staphylococcal evolution

MRSA was generated when methicillin-susceptible *Staphylococcus aureus* (MSSA) exogenously acquired *mecA* gene that was carried by a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). We searched for the origin of *mecA* gene by studying the genome of non-*aureus* staphylococcal species. By taking advantage of close linkage of *mecA* gene with a truncated copy of *mvaS* gene, which is an essential gene for the bacteria, we could identify the chromosomal region of *S. fleuretti* from where *mecA* gene originated. During the co-evolution of staphylococci and mammalian hosts, *mecA* gene was deleted out from the chromosome presumably due to the lack of selective pressure from antibiotic-producing microorganisms in the host animals. The situation was changed when humans started to use antibiotics as chemotherapeutic agents since 1940s, then *mecA* gene was re-introduced as a mobile genetic element called SCC*mec* into the chromosome of staphylococci colonizing humans and animals. Therefore, the natural direction of staphylococcal evolution was from methicillin-resistant to methicillin-susceptible, whereas, humans reversed the direction of evolution by introducing beta-lactam antibiotics in 1940s as chemotherapeutic agents.

II. Regulatory mutation: rifampin resistance promotes vancomycin resistance

Acquisition of the Vancomycin-intermediate *S. aureus* (VISA) phenotype has been another direction of the evolution of *S. aureus*. We have been studying the genetic mechanism underlying the VISA phenotype using the first VISA isolate Mu50 and its precursor strain Mu3. Mu3 represents hetero-VISA (hVISA), which was isolated from Juntendo University Hospital (JUH) prior to the isolation of Mu50. Both strains share the same PFGE pattern and a mutation in the sensor kinase gene *vraS* of *vraSR* two-component regulatory system (TCRS) that positively regulates cell-wall peptidoglycan synthesis. The *vraSR* TCRS is constitutively activated due to the mutated *vraS*, (designated *vraS**). As a result, cell-wall synthesis is enhanced, causing the thickening of the cell-wall peptidoglycan layers to prevent the access of vancomycin molecules to its target of action on the cytoplasmic membrane. In Mu50, the second mutation was identified in another TCRS *graRS*. Introduction of the mutated *graR*, designated *graR**, into Mu3 under the *tetL* promoter of a plasmid raised vancomycin resistance. Therefore, we considered that altered expression of two global regulators were sufficient for the VISA phenotype of Mu50. However, subsequent confirmatory experiment by introducing a single *graR** into the chromosome did not convert Mu3 to VISA. This prompted us to look for another mutation in Mu50 that promotes VISA phenotype in the presence of a single copy of *graR**. We found that the *rpoB* gene of Mu50 was mutated, designated *rpoB*(H481Y), with its 481st Histidine substituted by Tyrosine. The introduction of *rpoB*(H481Y) together with *graR** successfully converted Mu3 to VISA having the same level of vancomycin resistance with Mu50. We are now trying to understand the mode of activity of the *rpoB* mutation in the promotion of vancomycin resistance. Our study inidicated that it works at least partially by adjusting the drastic physiological changes caused by the two global regulator mutations; i.e., by recovering the disturbed homeostasis of the cell. Not only *rpoB*(H481Y) but many other *rpoB* mutations also were found to raise vancomycin resistance. About half (5/11) of the vancomycin-resistant mutant strains established from the subpopulations of hVISA strain Mu3*graR** carried *rpoB* mutations. Therefore, *rpoB* mutations constituted the characteristic shape of the hVISA population curve. Since *rpoB* mutants are easily obtained by exposing staphylococcal cells to rifampin, we obtained 10 rifampin-resistant mutants from each of the 9 clinical MRSA isolates in JUH. Out of the 90 rifampin-resistant mutants thus obtained, 86 (96%) showed decreased susceptibility to vancomycin in various degrees. Our experimental results raised a serious concern about the clinical use of rifampin against MRSA infection.