Staphylococcus aureus: a model of microbial adaptation to antibiotics

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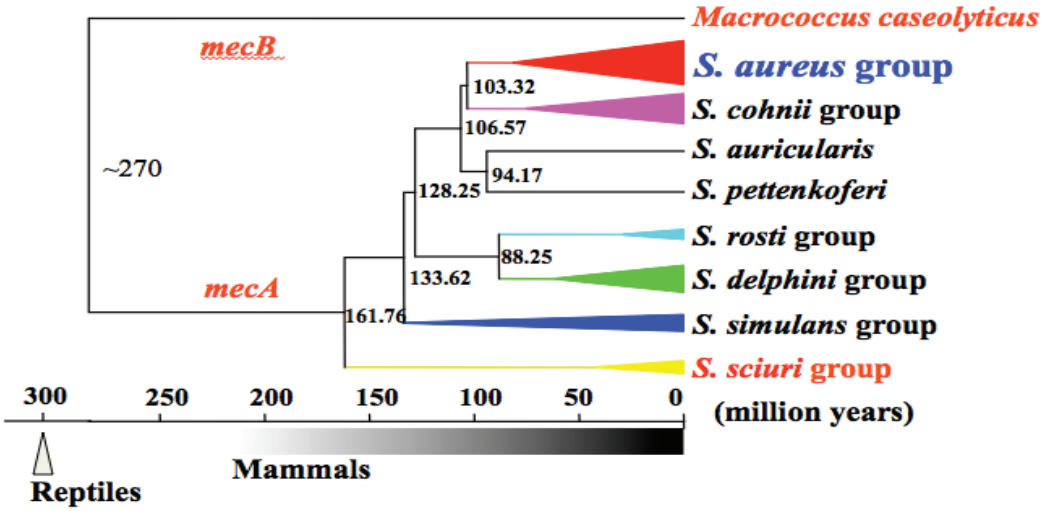
Among various pathogenic bacteria, *Staphylococcus* (*S.*) *aureus* has been the most versatile in the antibiotic resistance acquisition. MRSA is a subspecies of *S. aureus* that became resistant to all the beta-lactam antibiotics so far introduced into clinical use. *S. aureus* also conquered the assault of vancomycin, daptomycin and even linezolid. For the present, we do not have any single totally reliable chemotherapeutic agent against *S. aureus* infection.

The most remarkable mechanism of resistance that *S. aureus* has disclosed us is the acquisition of *mecA* gene from outside the cell. The *mecA* gene encodes MecA, a ß-lactam-resistant PBP (penicillin-binding protein). This horizontal gene transfer seems to occur quite frequently among staphylococcal species using SCC*mec* (staphylococcal cassette chromosome *mec*), a specially developed vehicle for the acquisition of useful genes from outside the cell. We found *S. fleurettii,* a commensal staphylococcal species of mammalians, is the origin of *mecA* gene. *S. fleurettii* belongs to *sciuri* group of *Staphylococcus*, the oldest staphylococcal species that are considered to have appeared about 200 million years ago. From the group more than 40 staphylococcal species branched off during the 200 million years of co-evolution of staphylococci and mammalians (Figure 1).

Figure 1 Emergence and coevolution of staphylococcal species with mammalians



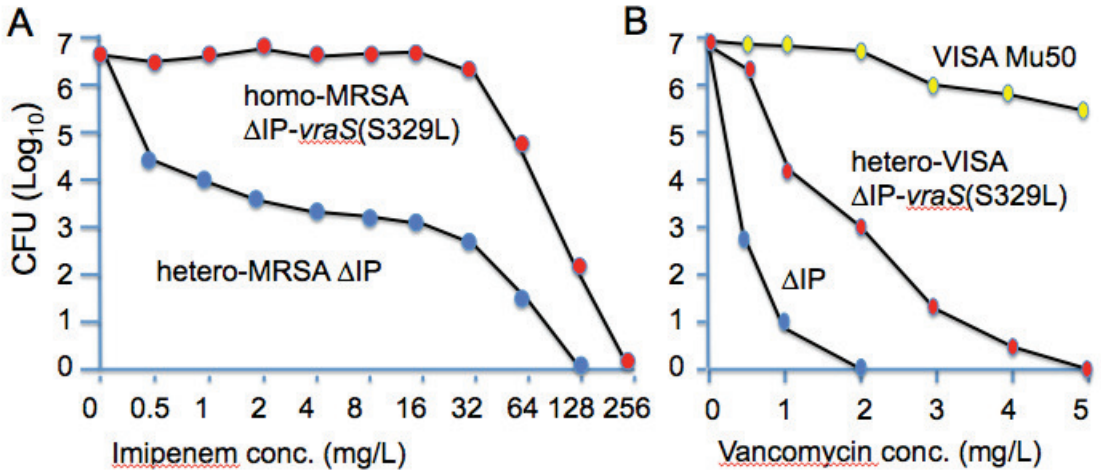
The oldest group of staphylococcal species carried *mecA* as an integral component of their chromosomes. With the lapse of time, however, *mecA* disappeared from their chromosome, presumably because the parasitized staphylococci became protected from antibiotic-producing micro-organisms by the immune system of the host animals. After the year 1960, when methicillin came into practical use, the colonizing bacteria had to reuse *mecA* gene by acquiring SCC*mec* from outside the cell.

The above story connotes that, before the advent of mammalian species, ancestors of *staphylococci* were frequently exposed to antibiotic-producing microorganisms in the nature. *Macrococcus (M.) caseolyticus*, found frequently in chicken, is such a bacterial speices that branched off the ancestor of *Staphylococcus* species about 300 million years ago. We found that some *M. caseolyticus* strains are resistant to ß-lactam antibiotics and possess a *mecA* gene homologue, designated *mecB* (Fig. 1). The *mecB* gene is transferable as a transposon, and found in both chromosomes and plasmids of *M. caseolyticus* strains. The *mecB* gene has not been found in *Staphylococcus* species. There seems to be a certain barrier for horizontal transfer across macrococcal and staphylococcal species.

The commensal bacteria found in the gut of animals such as *enterococci* should also be frequently exposed to the antibiotic-producing micro-organisms taken up together with the food. Therefore, it is understandable that some of the *Enterococcus* PBPs have low-binding affinities towards ß-lactam antibiotics. Together with staphylococcal MecA and macrococcal MecB, the enterococcal PBPs constitute the MecA super family.

Once *mecA* is acquired and transcribed, the *S. aureus* cell expresses a moderate level of ß-lactam resistance called heterogeneous (hetero-) methicillin resistance (hetero-MR). Probably the cell, called hetero-MRSA, is resistant enough to cope with the ß-lactam antibiotics produced by microorganisms in nature. However, some of the clinical hetero-MRSA strains were challenged with ß-lactam antibiotics having extremely strong anti-microbial activity such as imipenem. The outcome was unfavorable. We learned that hetero-MR can easily be converted to homogeneously highly resistance (homo-MR) to which no ß-lactam is effective (Fig. 2A). This change is brought about by spontaneous mutation. Mutations of a regulatory system *vraSR* are known to cause this conversion. The system up-regulates the genes involved in cell-wall peptidoglycan synthesis. Activated synthesis of peptidoglycan together with the expression of ß-lactam-insensitive PBP MecA make the cell-wall resistant to the assault by even the strongest of human-made ß-lactam antibiotics. Besides *vraSR*, hetero-to-homo conversion of ß-lactam resistance is also achieved by mutations of *rpoB* gene that encodes the ß subunit of RNA polymerase holoenzyme. In vancomycin resistance, the *vraS* mutation confers heterogeneously vancomycin-intermediate resistance (hetero-VI) on *S. aureus* (Fig. 2B). Curiously, the change from hetero-VI to VI phenotype (hetero-VI-to-VI conversion) is brought about by some *rpoB* mutations. The most popular one is *rpoB*(H481Y) that is also a well-kown mutation for rifampin resistance.

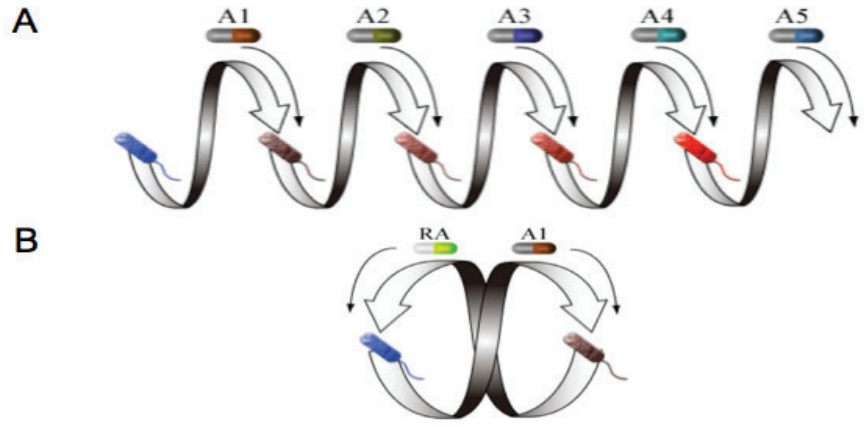
Figure 2 A mutation *vraS*(S329L) causes hetero-to-homo conversion of imipenem resistance and confers hetero-VISA phenotype on hetero-MRSA strain ΔIP



Another implication of the above hypothesis of the author is that not only antibiotics but also the resistance genes were already present in the nature before the mass use of antibiotics by humans. That is, emergence of antibiotic resistance is not a recent occurrence but was already there in soil or water 200 million years ago. Extending this view, the author thought there must be soil bacteria that produce 'anti-antibiotic-resistant-bacteria antibiotic'. Using *S. aureus* strains Mu50 and FDA209P as targets, the authors started screening Actinobacteria cultures. Mu50 is a multiply antibiotic-resistant MRSA strain against quinolone, vancomycin, rifampin etc. FDA209P is an antibiotic-susceptible reference *S. aureus* strain. By screening about 2,000 cultures we found one showing a queer activity. The culture contained a substance that has a strong activity against Mu50 (MIC=0.125 mg/L) and a week activity against FDA209P (≥64 mg/L). The substance was identified as Nybomycin, an old antibiotic reported in 1955. We found that the antibiotic is a DNA gyrase inhibitor. However, it did not inhibit wild-type DNA gyrase to which quinolones are effective. Instead, it specifically inhibited a mutated and quinolone-insensitive DNA Gyrase. Therefore, the activity of Nybomycin and Quinolones was opposite to each other. Since quinolone compounds are also present in natural products, it is plausible to postulate that Nybomycin was raised by an Actinobacterium to cope with naturally occurring quinolone-resistant bacteria. It is likely that the microorganisms on the earth had

already come into a dynamic equilibrium through perpetual conflicts menacing each other with antibiotics and protecting themselves with resistance genes before the advent of humans.

Figure 3 Endless vicious cycle between antibiotics and bacteria can be cut off by using Reverse Antibiotics (RA)



It should be noted that, although at extremely low frequency, Nybomycin-resistant mutants do emerge by selecting quinolone-resistant *S. aureus* strains with nybomycin. However, they turned out to be susceptible to quinolones. This reversion of quinolone susceptibility was due to the back mutation of the *gyrA* mutation. Thus, Nybomycin was designated 'Reverse Antibiotic (RA)' for quinolone resistance. RA is a novel category of antibiotics. Suppose a patient is infected with a pathogen that happened to be resistant to antibiotic A. RA can treat the patient but would leave RA-resistant mutants. However, antibiotic A is now effective against the RA-resistant mutants. Therefore, RA can kill most of the cells resistant to antibiotic A, and cure the residual cells of their resistance to antibiotic A in exchange for the resistance to itself (Fig. 3). RA is an embodiment of the wisdom of nature. By using RA, we would be released from the fate of continuous supply of new antibiotics to cope with the limitless ability of pathogens to raise antibiotic resistance (Fig. 3).