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Novel Mutation Sites in the Development of Vancomycin-Intermediate Resistance in *Staphylococcus aureus*

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Increased use of vancomycin has led to the emergence of vancomycin-intermediate *Staphylococcus aureus* (VISA). To investigate the mechanism of VISA development, 39 methicillin-susceptible strains and 3 MRSA strains were treated with vancomycin to induce non-susceptibility, and mutations in six genes were analyzed. All the strains were treated with vancomycin *in vitro* for 60 days. MICs were determined by the agar dilution and *E*-test methods. Vancomycin was then removed to assess the stability of VISA strains and mutations. Following 60 days of vancomycin treatment *in vitro*, 29/42 VISA strains were generated. The complete sequences of *rpoB*, *vraS*, *graR*, *graS*, *walK*, and *walR* were compared with those in the parental strains. Seven missense mutations including four novel mutations (L466S in *rpoB*, R232K in *graS*, I594M in *walk*, and A111T in *walR*) were detected frequently in strains with vancomycin MIC $\geq$ 12 μg/mL. Jonckheere-Terpstra trend test indicated these mutations might play an important role during VISA evolution. After the vancomycin treatment, strains were passaged to vancomycin-free medium for another 60 days, and the MICs of all strains decreased. Our results suggest that *rpoB*, *graS, walk*, and *walR* are more important than *vraS* and *graR* in VISA development.

Keywords: ***Staphylococcus aureus*, vancomycin, drug-resistance, mutations, jonckheere-terpstra trend test**

INTRODUCTION

Multiple antibiotic resistant *Staphylococcus aureus* continues to be one of the most common pathogens of both hospital-associated and community-associated infections worldwide (Klevens et al., 2007; Popovich et al., 2007; Hidron et al., 2008; Kallen et al., 2010). Methicillin-resistant *S. aureus* (MRSA) infection, acquired immunodeficiency syndrome (AIDS) and viral hepatitis B are the three major infectious diseases worldwide and pose a serious threat to public health (Dantes et al., 2013). Vancomycin is the first-line antibiotic therapy for MRSA infections (Sieradzki et al., 1999; Deresinski, 2005; Moellering, 2005). However, increased use of vancomycin has led to the emergence of vancomycin-intermediate *S. aureus* (VISA) (Hiramatsu et al., 1997b). Currently, the Clinical Laboratory Standards Institute (CLSI) categorizes *S. aureus* as vancomycin susceptible (VSSA) (MIC $\leq$ 2 μg/mL), vancomycin intermediate resistant (4–8 μg/mL), and vancomycin resistant (VRSA) (MIC $\geq$ 16 μg/mL) (Patel, 2014). VISA has been reported more frequently worldwide and