TITLE: RESISTANCE IN *IN VITRO*-SELECTED TIGECYCLINE-RESISTANT MRSA IS MEDIATED BY MepR PLUS MepA ALTERATIONS WITH NO CHANGES IN S10 PROTEIN

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a threatening nosocomial pathogen. One of the most recent options for the treatment of its infections is tigecycline, a modification of minocycline aiming to evade the main tetracycline's resistance mechanisms. The objective of this work was to determine whether the occurrence of resistance in *in vitro*-selected tigecycline resistant (TGC-R) MRSA strains could be mediated by unknown mechanisms. A tigecycline susceptible (TGC-S) ST5 clinical MRSA strain (MIC = 0.125 mg/L) was cultured in increasing levels of tigecycline until the resistance was reached (MIC = 1 mg/L), in triplicate. The genomes of the resistant, some intermediate, and the parental strains were sequenced to review the alterations that resulted in tigecycline resistance. Doubling times for the sequenced strains were determined. We also searched some MRSA genomes for modifications in the *rpsJ* gene coding for the S10 ribosomal protein, commonly associated with tigecycline resistance in both Gram-positive and Gram-negative pathogens. *In vitro*, tigecycline resistance was observed to be mediated by first, loss-of-function mutation in *mepR*, coding for a transcriptional regulator, resulting in derepression of *mepA*, coding for an efflux pump, as observed by qPCR experiments. Increased levels of tigecycline resistance were then obtained by successive mutations in *mepA* itself. Efflux of tigecycline was confirmed measuring the tigecycline MIC in the presence of the efflux pump inhibitor verapamil, resulting in up to 8-fold diminution of tigecycline MIC for the TGC-R strains. This evolutionary path to resistance was found in all 3 independent experiments, although the precise mutations occurring varied. It is important to note that no significant difference was detected when comparing the doubling times of the TGC-S and TGC-R strains, showing that the resistance to tigecycline does not imply a fitness cost to the cell. No alterations in the S10 protein were observed in the *in vitro*-generated TGC-R strains, but a K57M substitution, which has been associated with TGC resistance, was detected in clinical TGC-S strains. Thus, the primary pathway to tigecycline resistance in this CC5 branch of the species *in vitro* is derepression of the *mep* operon as the result of *mepR* loss-of-function mutation, followed by amino acid substitutions in the MepA efflux pump. Furthermore, mutations in the S10 protein are not always causative of tigecycline resistance in MRSA.

Keywords: MepA, MepR, MRSA, resistance mechanism, tigecycline

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