Title: TIGHTENING THE NOOSE ON TIGECYCLINE RESISTANCE IN *ENTEROCOCCUS FAECALIS*

Authors Navais, R. [1], Dabul, A.N.G. [1], Gilmore, M. S. [2], Camargo I.L.B.C. [1]

Institution [1] IFSC/USP – São Carlos Institute of Physics/University of São Paulo (1100 Avenida Joao Dagnone, 13563-120, Sao Carlos – SP), [2] Department of Ophthalmology (Microbiology and Immunobiology), Harvard Medical School, Massachusetts Eye and Ear Infirmary (243 Charles St, Boston, MA 02114, USA)

Abstract:

Different approaches have been adopted to elucidate the mechanisms responsible for tigecycline-resistance in *Enterococcus faecalis*. To this purpose, we have examined three *in vitro*-generated tigecycline-resistant mutant strains as well as tigecycline-susceptible and tigecycline-resistant clinical isolates. It is known that tigecycline interacts with six nucleotides of the 16S rRNA in the A site of the ribosome. In addition, a decrease in tigecycline susceptibility has been observed in *Escherichia coli* strains with known mutations in the 16S rRNA. We now know that the tigecycline-resistance phenotype shown by our clinical isolates is not caused by a mutation in this gene, as revealed by the sequencing of an internal fragment of the 16S rRNA gene harbouring the nucleotides that form the tigecycline binding site. A flavin-dependent monooxigenase, TetX, that oxidises tetracycline to inactive products, has been found in bacteria of the genus *Bacteroides*. Furthermore, it has been reported that tigecycline is a substrate for TetX and that bacterial strains containing the *tetX* gene are resistant to tigecycline. Therefore, a PCR-based screening of gene *tetX* was performed on our clinical isolates, which directed us to look elsewhere to explain the observed tigecycline-resistance phenotype. With respect to the *in vitro*-derived tigecycline-resistant mutant strains, the significant decrease in tigecycline MIC in the presence of efflux pump inhibitor verapamil shown by two of them, suggests an efflux pump may be involved in tigecycline-resistance. Further, the *in vitro* generation of stable tigecycline-resistant strains allow us to affirm that tigecycline-resistance in *E. faecalis* can be caused by genetic mutation. It remains to be seen whether resistance can also be caused by acquiring resistance determinants from another bacterium. The comparative genome analysis of three tigecycline-resistant and two tigecycline-susceptible clinical isolates should help answer this question. In conclusion, although we have ruled out some plausible hypotheses, the mechanisms of resistance to tigecycline in *E. faecalis* remain to be elucidated. We expect that the sequencing and comparative genome analysis of our strains and the experiments we have planned to carry out will shed light on this issue.

Keywords: *Enterococcus faecalis*, resistance to tigecycline

Support: Capes - process 88887.063842/2014-00