Title: INSIGHTS ON THE TIGECYCLINE RESISTANCE MECHANISM IN MRSA

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Abstract:

*Staphylococcus aureus* is an opportunistic pathogen that is remarkable in its capability to develop resistance to antimicrobials. During an epidemiological study, we found that among 36 methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients at a hospital in Belo Horizonte, 13 were resistant to tigecycline, even though the drug was not in use at the hospital. All tigecycline-resistant MRSA were classified as ST105. Five of them (belonging to the same pulsotype) were selected to be studied with the goal of elucidating the underlying tigecycline resistance mechanism. PCR was used to examine these strains for previously described genes tetX and tetX1 that could confer such a phenotype. The minimal inhibitory concentration to tigecycline in the presence of efflux pump inhibitors was determined to test whether resistance could be associated to efflux pumps. Additionally, *in vitro* selection of tigecycline-resistant mutants of a susceptible strain (MIC = 0.25 mg/L) of the same pulsotype was undertaking to try to recapitulate the development of resistance under controlled conditions, and finally, the growth curves of the all the strains (clinical and those generated *in vitro*) were analyzed for differences in growth profile. None of the isolates harbored known genes tetX or tetX1, and the MIC of these strains to tigecycline was not affected by the efflux pump inhibitors tested, which suggests that the mechanism of resistance to tigecycline in MRSA is divergent from what is known. *In vitro* selection of a tigecycline-resistant strain was performed in triplicate, and in one experiment we were able to isolate a resistant strain after 4 days (MIC = 1 mg/L). After 10 days, strains with MIC = 4 mg/L were isolated from the 2 additional parallel experiments. Growth curves showed more variation among clinical isolates than between the strains selected *in vitro*, as expected. In general, the generation time did not change over time for mutants selected *in vitro*, indicating that at least under laboratory growth conditions, there is little fitness cost associated with development of resistance to tigecycline. To understand the microevolution of tigecycline resistance, strains from each parallel *in vitro* experiment are being sequenced. Thus, we were able to discount known mechanisms of resistance to tigecycline in MRSA and are now focusing studies on changes in genome sequence to elucidate the resistance mechanism.

Keywords: MRSA, tigecycline resistance, resistance mechanism

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