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Paper Poster Session
Resistance mechanisms in staphylococci

Adaptation to vancomycin pressure of heterogeneously vancomycin-resistant Staphylococcus aureus

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Background: The mechanisms behind the emergence of heterogeneous vancomycin resistance (hVR) in *Staphylococcus aureus* are only partially understood. From a microbiological standpoint, two concurrent hypotheses regarding hVR can be proposed. First, hVR could reflect an enhanced potential to adapt to vancomycin, as shown previously in hVR *Staphylococcus capitis*. This implies that hVR strains could increase MICs more rapidly when challenged with vancomycin, and serve as a reservoir for the emergence of more resistant strains. Second, hVR could result from previous selection by or adaptation to glycopeptides or other stress sources, without implying a better adaptation potential nor the ability to eventually reach higher MICs under treatment. We tested these competing hypotheses using an *in vitro* model of sustained vancomycin selection pressure.

Material/methods: hVR was tested quantitatively using the population analysis profile-area under the curve (PAP-AUC) reference method and defined qualitatively as an AUC >90% of the hVR prototype strain Mu3. Thirty-six hVR and non-VR clinical *S. aureus* strains (n=12 and 24, respectively) and the control strains Mu3 and ATCC29213 (vancomycin-susceptible), all with vancomycin MICs < 2mg/L, were included. Broth-microdilution (BMD) vancomycin MICs were determined daily in 96-well plates during 15 days in two independent experiments. We used 1.5-fold geometric dilution series to improve MIC resolution. Each day, bacteria harvested from the well with the highest vancomycin concentration and visible growth were used to inoculate the next BMD MIC plate. Daily log-MICs were regressed on time to estimate the slopes of MIC increase. Linear correlation between log-MICs and log-AUCs was computed at each time point to examine how the association between hVR and vancomycin MIC evolved during treatment.

Results: Initial vancomycin MICs were 1.4-fold higher in hVR strains compared to non-hVR strains ($P < .05$). Regression slopes of log-MICs on time were significantly higher in non-hVR strains ($P < .01$). The correlation between log-AUCs and vancomycin MICs decreased rapidly ($P < .05$) and reached zero after 6 days. All strains acquired vancomycin resistance (MIC range 3-6 mg/L) after 15 days. Final MICs were not significantly different between hVR and non-hVR strains.

Conclusions: The hVR phenotype in *S. aureus* is associated with moderately increased pre-treatment MICs but not with an enhanced ability to increase MICs under vancomycin treatment or to reach higher post-treatment MICs *in vitro*. These findings disfavor the hypotheses that hVR reflects an enhanced adaptation potential or that this phenotype is qualitatively different from a moderately

increased MIC. Thus, from an operational standpoint, hVR could be regarded as a proxy measure of vancomycin resistance, endowed with a higher resolution and a lower clinical breakpoint than usual MIC methods. This interpretation might help explain why several clinical studies failed to identify links between hVR and vancomycin treatment failure in *S. aureus*.