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Adaptation to vancomycin pressure of heterogeneously vancomycin-resistant *Staphylococcus aureus*

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Introduction

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.

Materials and Methods

Quantitative evaluation of hVR

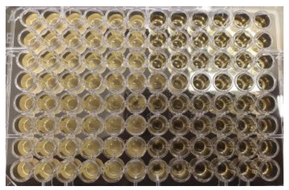
Population analysis profile-area under the curve (**PAP-AUC**) = reference method → hVR was defined qualitatively as an AUC >90% of the hVR prototype strain Mu3

Strains: 36 clinical *S. aureus* strains, all having vancomycin MICs <2mg/L

- 12 **hVR** *S. aureus*, confirmed by PAP-AUC
- 24 **non-hVR** *S. aureus* (vancomycin-susceptible *S. aureus*, VSSA):
  - 12 with initial suspicion of hVR but non confirmed
  - 12 fully-susceptible to glycopeptides (MICs <0.5mg/L)

Controls: Mu3 (prototype hVR) and ATCC29213 (VSSA)

Exposition to vancomycine pressure



Broth-microdilution (BMD) vancomycin MICs in Brain Heart Infusion were determined daily in 96-well plates during 15 days in 2 independent experiments. MIC resolution was improved using 1.5-fold geometric dilution series.

Each day, bacteria harvested from the well with the highest vancomycin concentration and visible growth were used to inoculate the next BMD MIC plate.

Data analysis

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 - Discussion

Initial vancomycin MICs were 1.4-fold **higher** in **hVR** strains compared to non-hVR strains ($P < 0.05$).

Regression slopes of log-MICs on time were significantly higher in non-hVR strains ($P < 0.01$). The correlation between log-AUCs and vancomycin MICs decreased rapidly ($P < 0.05$) and reached zero after 6 days.

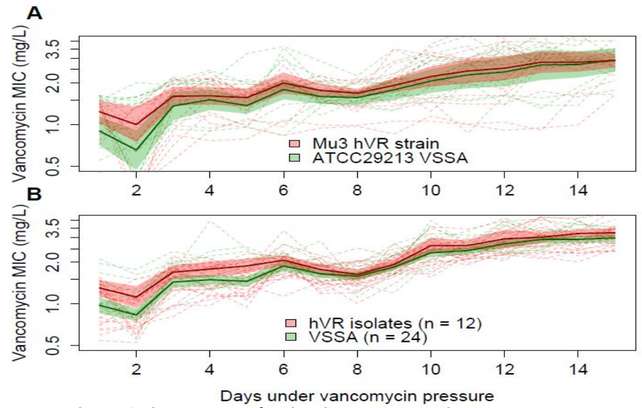


Figure 1. Vancomycin MICs increase under *in vitro* vancomycin pressure. *S. aureus* control strains Mu3 and ATCC29213 (**A**), and 36 clinical isolates either hVR or VS (**B**), were subcultivated daily in vancomycin-supplemented medium. Vancomycin MICs increased with time and were on average larger than the 2 mg/L susceptibility breakpoint after 15 days. Dashed lines denote individual replicates for control strains or geometric mean of at least 2 independent experiments for clinical strains. Vertical jitter added for readability. Thick solid lines denote geometric means, colored areas denote 95% CI of the geometric mean.

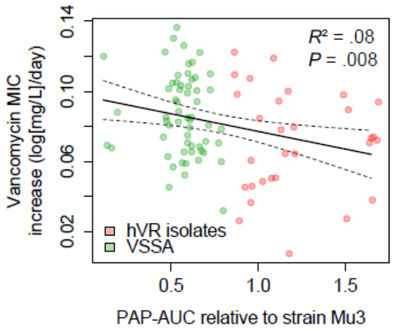


Figure 2. VSSA strains increase vancomycin MICs faster than hVR strains under vancomycin pressure. For clinical isolate (n = 36) in each of at least 2 independent experiments, log-MICs were regressed on time to obtain estimates of the kinetics of resistance acquisition (**A**). The MIC increase was faster in VSSA strains as compared to hVISA, suggesting that initially susceptible strains are able to adapt quickly to vancomycin pressure. Regression coefficients of log-MIC on PAP-AUC were computed (controlling for inter-experiment variation) at each time point to monitor the predictive power of PAP-AUC regarding MIC increase under vancomycin pressure.

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.

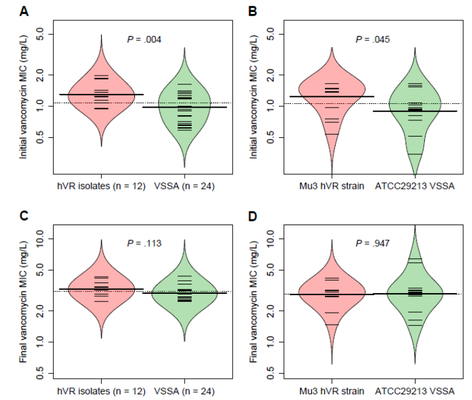


Figure 3. Vancomycin-heteroresistant isolates have larger vancomycin MICs before, but not after, 15 days of vancomycin pressure. Shown are comparisons of the vancomycin MICS of 36 clinical *S. aureus* strains (**A, C**) and control strains (**B, D**) before and after vancomycin pressure experiment. hVR strains exhibited larger MICs on day 1 (**A, B**), however final MICs were comparable between hVISA and VSSA strains (**C, D**). *P*-values are Welch's *t*-tests on log-transformed data.

Conclusion

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*.