Detection of *Staphylococcus aureus* isolates with intermediate vancomycin susceptibility (VISA) and heteroresistance (hVISA) remains problematic. Population analysis profile-area under the curve ratio (PAP-AUC) is the gold standard, with the level of resistance of an isolate expressed as a ratio of the AUC of growth at various vancomycin concentrations compared to a reference hVISA strain (Mu3) (1). By convention, an AUC ratio of 0.9 is taken as a cutoff for hVISA, but there is no set value for a full VISA phenotype. In recently published papers (1, 2), we determined the vancomycin susceptibility profile of 75 putative VISA and vancomycin-susceptible *S. aureus* (VSSA) isolates by multiple methods: reference broth microdilution, Etest, and PAP-AUC (see Table S1 in reference 2). We defined 26 strains as VISA by Etest (vancomycin MIC, ≥3 μg/ml), whereas 47 were hVISA/VISA by PAP-AUC (reference broth microdilution was not considered further as doubling dilutions could not define strains with MICs at critical values from 2 to 4 μg/ml). As Fig. 1 shows, there was strong correlation between PAP-AUC and Etest values for each strain ($R^2 = 0.7$), and the PAP-AUC ratio of 1.15 was predicted to correspond to an Etest vancomycin MIC of 3 μg/ml.

We sequenced the genomes of the 75 VISA and VSSA strains and ascertained single nucleotide polymorphisms (SNPs), insertions, and deletions (2). We identified genes where mutations had been associated with the development of VISA based on a large collection of reported molecular genetic studies. We developed an informal model for predicting VISA (described in detail by Alam et al. [2]). An interactive version of the model is available online (https://tread.shinyapps.io/VISA-shiny/). For the 75 strains in our study, there was a positive correlation between the number of SNPs/indels called by the model and both the Etest MIC and PAP-AUC ratio to Mu3. In the case of the Etest data, the regression predicted that a genome containing an SNP would have a vancomycin MIC of 3 μg/ml. Based on defining VISA at an Etest MIC of 3 μg/ml, the model is 81.3% specific and 73.1% sensitive. However, using the PAP-AUC ratio to Mu3 of 0.9 (dashed line in Fig. 2a and b), the model is only 63.5% specific and 47.8% sensitive. If the threshold for VISA based on PAP-AUC is set instead to a ratio of 1.15 to Mu3, the specificity and sensitivity are 81.1% and 82.4%, respectively. At a ratio of 1.15, there are fewer strains classed as VISA that lack any mutation in one of the candidate genes. It is notable that 1.15 is the point closest to where the regression line for the model passes through 1 model SNP per strain. In the range of 1.15 to 1.35, high sensitivity and specificity are maintained (it is notable that VISA has been defined as a ratio to Mu3 of >1.3 by PAP-AUC by others [3]). If the threshold is raised above this range in our model, sensitivity tends to increase (as strains with PAP-AUC ratios of >1.15 are much more likely to contain SNPs) but specificity decreases.

The genomic model for VISA is far from complete and would benefit greatly from whole-genome sequencing of many more strains with a range of PAP-AUC ratios. However, here we illustrate how an underlying genetic model can be integrated into the
FIG 2 (a) PAP-AUC threshold for VISA at 0.9 (screenshot from https://tread.shinyapps.io/VISA-shiny/). (b) PAP-AUC threshold for VISA at 1.15 (screenshot from https://tread.shinyapps.io/VISA-shiny/). The top panel shows the accuracy of the prediction (true positive + true negative/all outcomes) and sensitivity (true positive/true positive + false negative). Bar plots show the distribution of the VISA/VSSA strain calls from the perspective of the model predictions (left) and true phenotypes (right).
difficult decision process of setting antibiotic resistance breakpoints.

REFERENCES

