

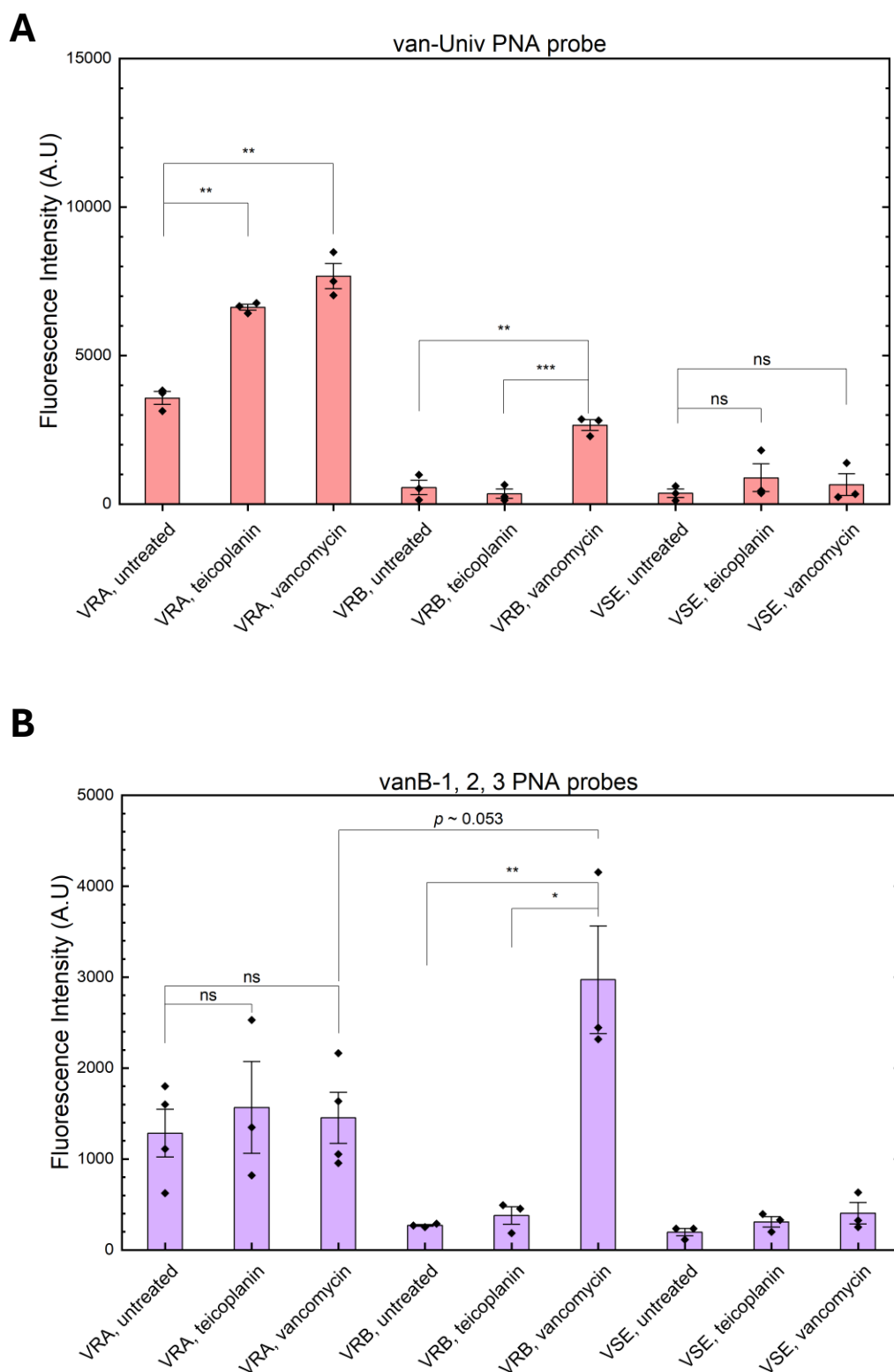


Figure S1. Fluorescence intensities of vancomycin-resistant and vancomycin-susceptible enterococci detected using PNA-FISH probes. (A) Fluorescence intensities obtained using the van-Univ PNA probe. **(B)** Fluorescence intensities obtained using the vanB-1, 2, 3 PNA probes. Bars represent mean fluorescence intensities, and error bars indicate standard errors from at least three independent biological replicates ($n = 3$). Individual replicate values are shown as black diamonds. Statistical significance was determined using two-sample t-tests. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

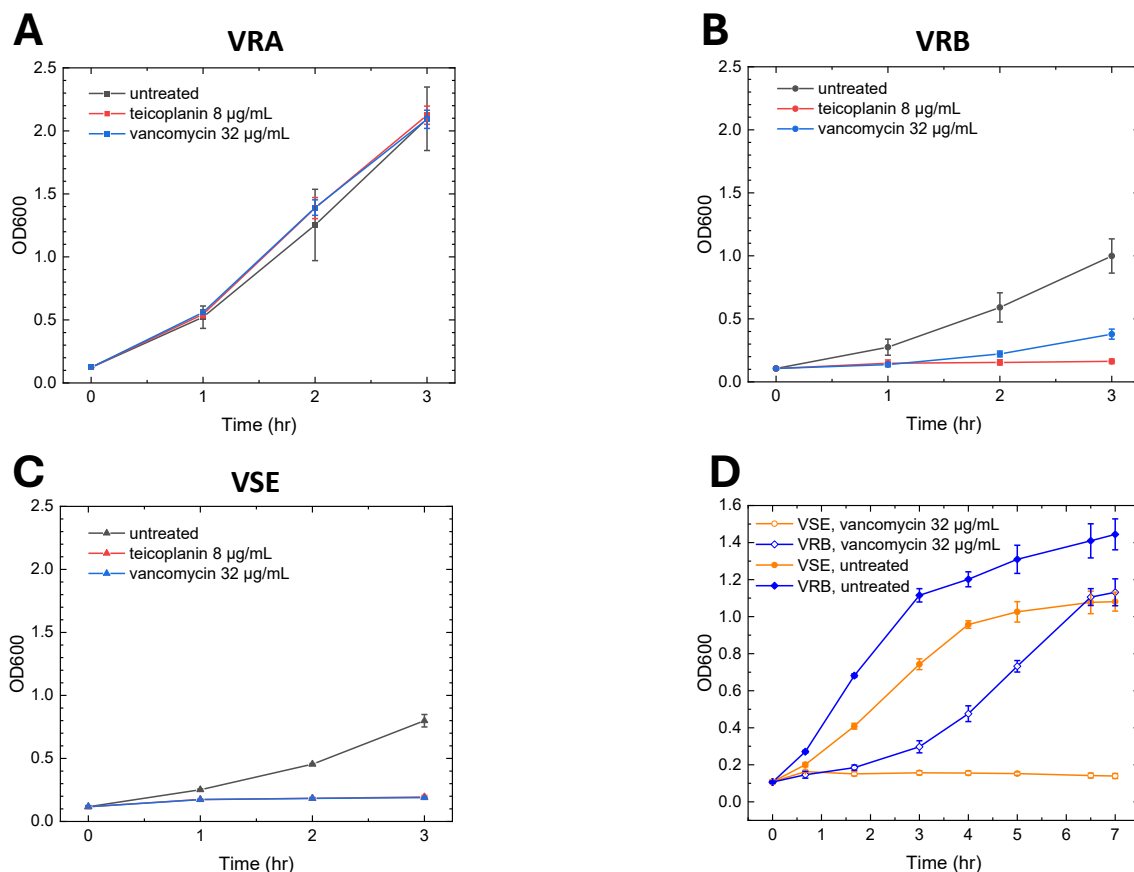


Figure S2. (A-C) Growth kinetics of *Enterococcus faecium* strains VRA, VRB, VSE, under treatment with teicoplanin or vancomycin. Optical density at 600 nm (OD_{600}) was measured at the indicated time points using a NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). VRA is resistance to both antibiotics, whereas VRB is resistant only to vancomycin. **(D)** Extended growth kinetics of VRB and VSE, showing that VRB continues to grow upon treatment with vancomycin and reaches a stationary phase at approximately 7 hr, whereas VSE exhibits only a transient increase in OD_{600} followed by growth inhibition. Data points represent mean OD_{600} values $\pm$ standard error of the mean from three independent measurements ($n = 3$).

vanB

ATGAATAGAATAAAAAGTCGCAATCATCTTCGGCGGTTGCTCGGAGGAACATGATGTGT
CGGTAAAATCCGCAATAGAAATTGCTGCGAACATTGATACGGAAAAATT**CGATCCGCAC**
TACATCGGAATTACAAAAAACGGTGTATGGAAGCTATGCAAGAAGCCATGTACGGAATG
GGAAGCCGACAGTCTCCCCGCCATACTCTCCCCGGATAGGAAAACGCAT**TGGGCTGCT**
TGTCATGAAAGAAAGCGAATACGAAACACGGCGTATTGATGTGGCTTTCCCGGTTTTG
CATGGCAAATGCGGGGAGGATGGTGCGATACAGGGGCTGTTT**GTATTGTCTGGTATC**
CCCTATGTGGGCTGTGATATTCAAAGCTCCGCAGCTTGCATGGACAAATCACTGG**CCT**
ACATTCTTACAAAAAATGCGGGGCATCGCCGTTCCCGAATTTCAAAT**G**ATTGATAAAGGT
GACAAGCCGGAGGCGGGTGCGCTTACCTACCCTGTCTTTGTGAAGCCGGGCACGGTC
AGGTTCTGTCCTTTGGCGTAACCAAAGTAAACGGTACGGAAGAACTTAACGCTGCGATA
GAAGCGGCAGGACAATATGATGGAAAAATCTTAATTGAGCAAGCGATT**TCGGGCTGTG**
AGGTCGGGTGTGCGGTCATGGGGAACGAGGATGATTTGATTGTGCGCGAAGTGGAT
CAAATCCGGCTGAGCCACGGTATCTTCCGCATCCATCAGGAAAACGAGCCGGAAAAA
GGCTCAGAAAATGCGATGATTACAGTTCCCGCAGACATTCCGGTCGAGGAACGAAAT
CGGGTGCAAGGAAACGGCAAAGAAAGTATATCGGGTGCTTGGATGCAGAGGGCTTGC
CCGTGTTGATCTTTTTTTGCAGGAGGATG**GCGGCATCGTTCTAA**ATGAGGTCAATACC
CTGCCCGG**T**TTTACATCGTACAGCCGCTACCCACGTAT**G**ATGGCCGCGCGCAGGAATC
ACGCTTCCTGCACTGATTGACAGCCTGATTACATTGGCGTTAAAGAGGTGA

Target site of van-Univ probe

Target site of vanB-1 probe

Target site of vanB-2 probe

Target site of vanB-3 probe

: Sequence checked by PCR product sequencing

: Nucleotide appearing as T from PCR sequencing

: Nucleotide appearing as A from PCR sequencing

: Nucleotide missing from PCR sequencing

: Possible stop codon position due to nucleotide deletion

vanA

ATGAATAGAATAAAAAGTTGCAATACTGTTTGGGGGTTGCTCAGAGGAGCATGACGTATC
GGTAAAATCTGCAATAGAGATAGCCGCTAACATTAATAAAGAAAAAATACGAGCCGTTATA
CATTGGAATTACGAAATCTGGTGTATGGAAAATGTGCGAAAAACCTTGCGCGGAATGG
GAAAACGACAATTGCTATTCAGCTGTACTCTCGCCGGATAAAAAAATGCACGGATTACT
TGTTAAAAAGAACCATGAATATGAAATCAACCATGTTGATGTAGCATTTTTCAGCTTTGCA
TGGAAGTCAGGTGAAGATGGATCCATACAAGGTCTGTTTGAATTGTCCGGTATCCCT
TTTGTAGGCTGCGATATTCAAAGCTCAGCAATTTGTATGGACAAATCGTTGACATACAT
CGTTGCGAAAAATGCTGGGATAGCTACTCCCGCCTTTTGGGTTATTAATAAAGATGATA
GGCCGGTGGCAGCTACGTTTACCTATCCTGTTTTTGTAAAGCCGGCGCGTTCAGGCT
CATCCTTCGGTGTGAAAAAAGTCAATAGCGCGGACGAATTGGAATACGCAATTGAATC
GGCAAGACAATATGACAGCAAAATCTTAATTGAGCAGGCTGTT**TCGGGCTGTGAGGTC**
GGTTGTGCGGTATTGGGAAACAGTGCCGCGTTAGTTGTTGGCGAGGTGGACCAAATC
AGGCTGCAGTACGGAATCTTTCGTATTCATCAGGAAGTCGAGCCGGAAAAAGGCTCT
GAAAACGCAGTTATAACCGTTCCCGCAGACCTTTCAGCAGAGGAGCGAGGACGGATA
CAGGAAACGGCAAAAAAATATATAAAGCGCTCGGCTGTAGAGGTCTAGCCCGTGTGG
ATATGTTTTTACAAGATAACGGCCGCATTGTACTGAACGAAGTCAATACTCTGCCCGGT
TTCACGTCATACAGTCGTTATCCCGTATGATGGCCGCTGCAGGTATTGCACTTCCCG
AACTGATTGACCGCTTGATCGTATTAGCGTTAAAGGGGTGA

Figure S3. *vanA* and *vanB* sequences from CARD. Regions highlighted in yellow show the sequences confirmed by sequencing PCR amplification products. Mutations are highlighted in distinct colors. Target sites of FISH probes are indicated in distinct colors. The van-Univ PNA probe shows perfect matches (magenta) on both *vanA* and *vanB*, while vanB-1/2/3 shows perfect matches only on *vanB*.