Prevalence of Vancomycin Variable Enterococci in the Netherlands and the mechanism of resistance induction

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Background

Vancomycin resistant enterococci (VRE) may cause difficult to treat infections in immunocompromised individuals.

However, enterococci may also harbour vancomycin resistance genes while being phenotypically susceptible; so-called vancomycin-variable enterococci (VVE).

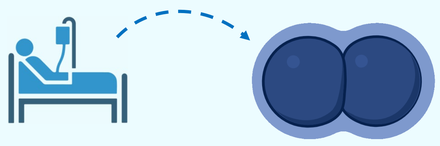
These VVE present laboratories with clinically significant challenges as these isolates may be missed by classic, culture-dependent methods.

Objective

Little is known about the prevalence of VVE in the Netherlands, as the national guidelines rely solely on phenotypic selection. To address this, two laboratories in the South-Eastern region of the Netherlands collaborated to investigate the presence of VVE in their facilities.

Methods

Sample collection



Vancomycin *susceptible* enterococci from infections.

N = 233 (Rijnstate)

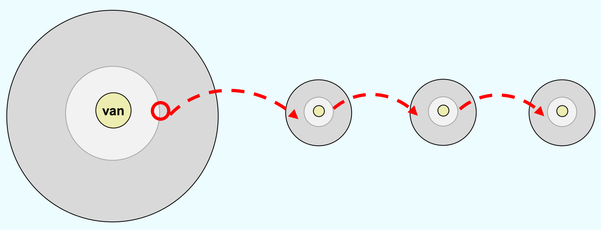
N = 228 (MUMC)

Screening for *van*-genes

qPCR for *vanA, vanB, vanC, vanD* genes.[1]

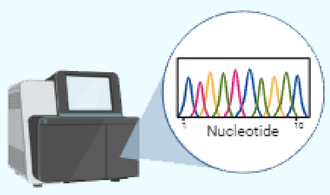
Resistance induction

4-6x consecutive sub-culturing *van*-gene positive isolates under Vancomycin pressure.



→ N = 3 Enterococci <u>before</u> resistance induction
→ Same N = 3 Enterococci <u>after</u> resistance induction

Characterisation of mutations



Whole Genome Sequencing[1] of *van*-gene positive Isolates.
-before resistance induction
-after resistance induction

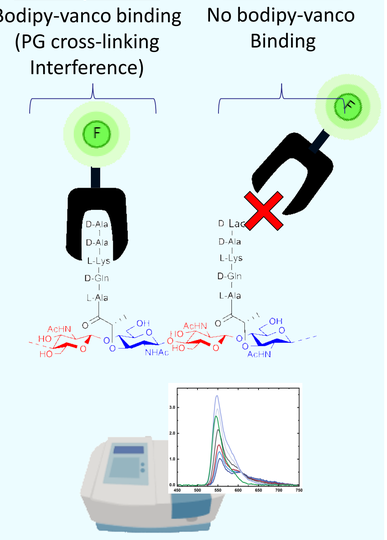
RNA analysis

-RNA extraction
-DNAse treatment
-qPCR *vanB* vs *recG* (housekeeping)

Vancomycin binding assay

<u>susceptible:</u>
Bodipy-vanco binding (PG cross-linking Interference)

<u>resistant:</u>
No bodipy-vanco Binding



Results

VVE prevalence

A total of 6 VVE were detected (1,3% of susceptible enterococci).

Resistance induction

Of these, 3 VVE developed vancomycin resistance *in vivo.*

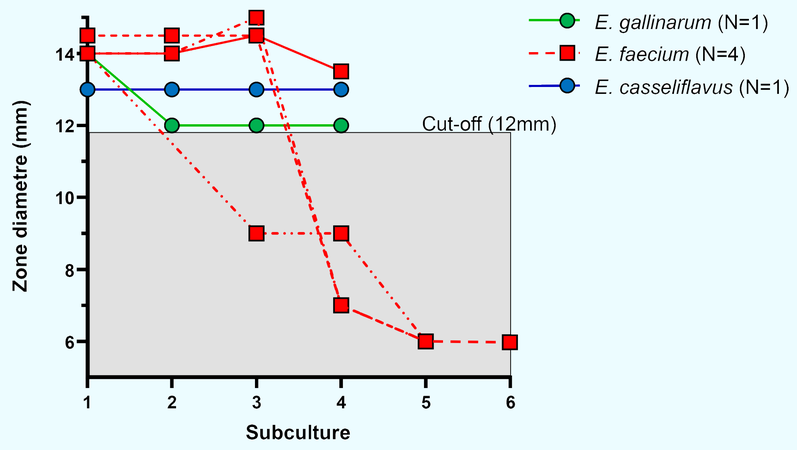


Figure 1: Vancomycin susceptibility results of the consecutively sub-cultured VVE.

Characterisation of mutations

These 3 isolates were clonally related, but each harbored a unique mutation in the *van*-operon after developing resistance: *vanR* T189K, *vanS* G253C & *vanS* L282V.

RNA analysis

No difference in RNA expression between susceptible and resistant phenotypes of the same VVE isolates.

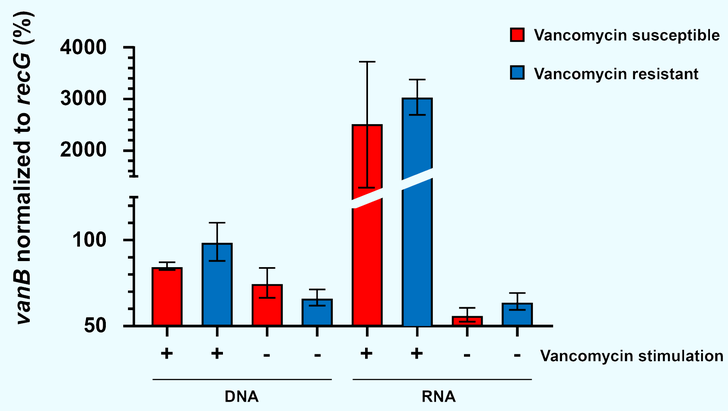


Figure 2: Relative DNA (left) and RNA levels (right) of vanB in the three VVE. Each in a vancomycin susceptible (red) and vancomycin resistant (blue) phenotype.

Vancomycin binding assay

The relative amount of vancomycin-BODIPY incorporated in the cell wall was lower in the resistant phenotype than in the susceptible phenotype (52.8%, p≤0.05).

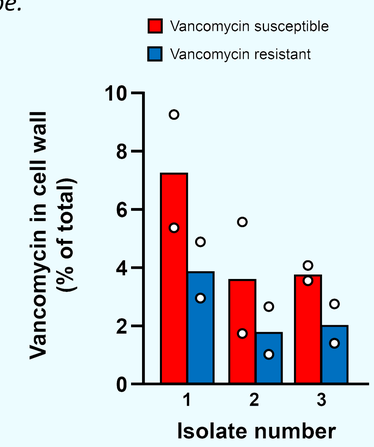


Figure 3: vancomycin binding in phenotypically resistant (blue) and susceptible (red) VVE. White circles: duplo measurements.

Conclusions

- The prevalence of vancomycin variable enterococci (VVE) seems to be low in The Netherlands.
- Some VVE (*E.faecium*) developed vancomycin resistance when exposed to antibiotic pressure *in vitro*, which illustrates the potential threat to infected individuals.
- We endorse the recommendation[2] for molecular resistance screening of invasive, phenotypically susceptible enterococci, if these are repeatedly cultured despite appropriate glycopeptide therapy.

References:
1. Flipse J, von Wintersdorff CJH, et al. Sci Rep. 2019;9(1):6949.
2. Sivertsen A, Pedersen T, et al. Antimicrob Agents Chemother.179 2016;60(7):4119-27.

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