Transfer of silenced *vanA* gene cluster by broad host range plasmid

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Objectives

The purpose of this study was to explain vancomycin resistance development in a nosocomial outbreak of vanA-containing, vancomycin susceptible enterococci within two hospitals in Trøndelag, Norway.

Materials & methods

48 E. faecium and one E. faecalis linked to a nosocomial outbreak and shown to be vanA-containing vancomycin susceptible or resistant enterococci (VSE/VRE) by vanA PCR and phenotypic testing, were included in the study. 42 of the E. faecium isolates were clonal by PFGE and six were unique. Four of the clonal vanA-containing VSEfm isolates and two VREfm isolated after vancomycin treatment of VSEfm infections were whole-genome sequenced by Illumina MiSeq. The plasmid content of all unique pulsotypes was analyzed with S1-nuclease PFGE and hybridizations. Transfer of vanA was assessed by filter mating. The VSE (n=35) were cultivated in BHI broth containing 8 mg/L vancomycin for in vitro development of VREfm. The vanA cluster composition was determined by contig gap closure with PCR and Sanger sequencing, and transcription profile was assessed with RT-qPCR.

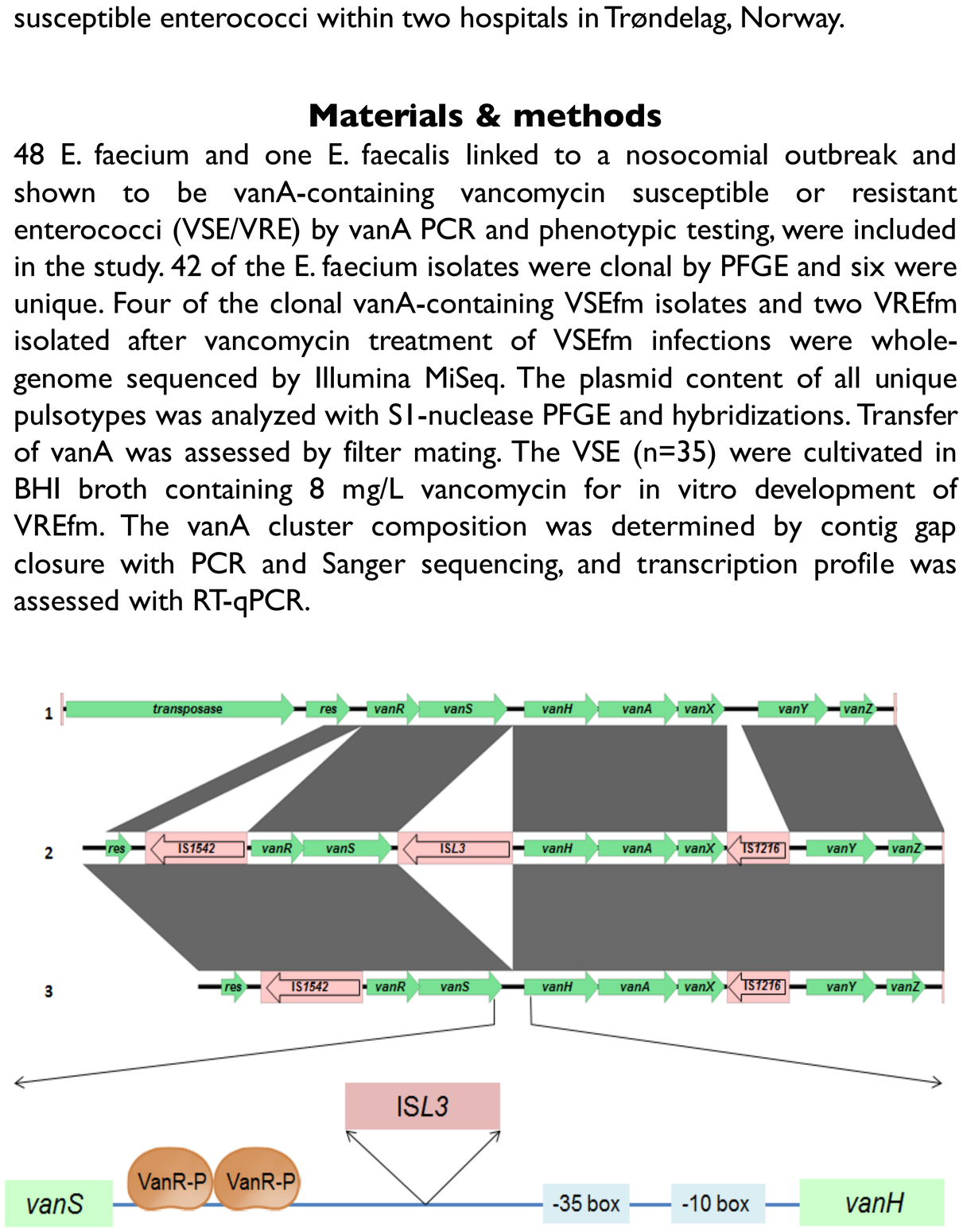

Figure 1: The insertion site of IS*L3* illustrated in a scaled alignment of *vanA* clusters from Norwegian clonal 2) VSEfm and 3) VREfm to reference 1) Tn*1546* (GenBank Acc. No. M92792). In the zoomed view, the location of IS*L3* between the binding site of the VanR activator (VanR-P) and *vanHAX* promoter (-35 and -10 boxes) is indicated.

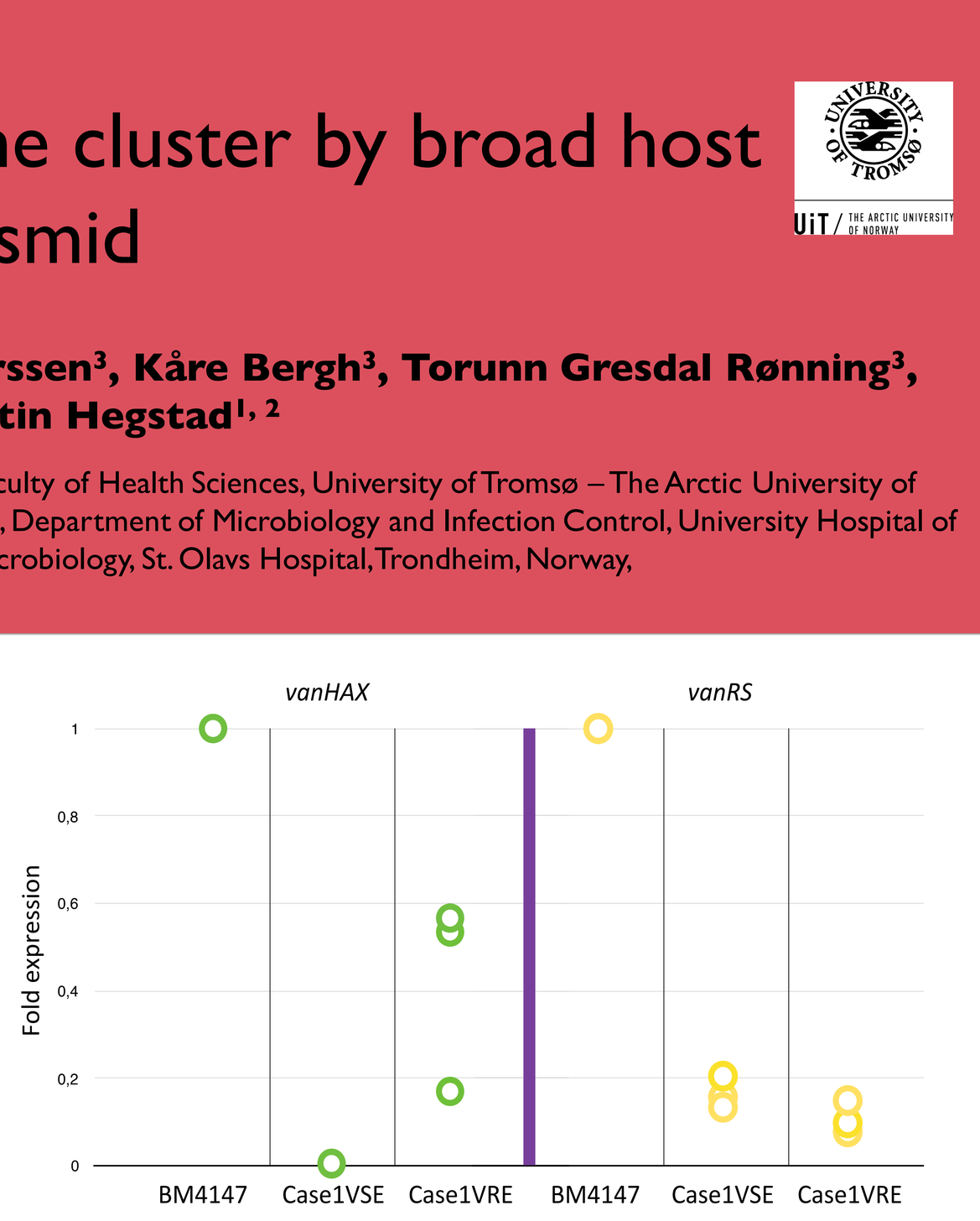

Figure 2. Expression levels of the *vanHAX* and *vanRS* operons in the VanA silenced (Case1VSE) and the VRE (Case1VRE) isolates relative to BM4147 (Tn*1546* prototype) assessed by RT-qPCR. Data points for three independent experiments are shown for each isolate. All measurements were normalized against house-keeping gene glutamate dehydrogenase (*gdh*).

Figure 3. Plasmid profiles shown by S1-nuclease restriction PFGE and by subsequent Southern blotting and hybridization with indicated probes. A: *Enterococcus* outbreak isolates representing the unrelated pulsotypes (lanes 4-9), as well as a vancomycin variable *E. faecalis* (lane 10). B: *E. faecium* transconjugants from Case1VSE (lane 12-14) using chloramphenicol or Case1VRE using vancomycin (lanes 15-17) selection agent to plasmid-free recipient 64/3 (lane 11). 1: BM4147 *vanA*$^+$ control. 2: Case1VSE. 3: *rep*$_{pIP501}^-$ control. The sizes of the molecular marker (M) are indicated.

Results

The only genetic difference between the *vanA* clusters of the VSE and VRE was an IS*L3*-family element upstream of *vanHAX* in the VSE of all *E. faecium* pulsotypes as well as in the *E. faecalis* isolate, which caused silencing of the essential *vanHAX* gene transcription (Fig. 1). All VSE and VRE isolates had insertions of IS*1216* in the intergenic region between *vanX* and *vanY* as well as IS*1542* between the resolvase gene and *vanR* as compared to the prototype *vanA* gene cluster.

Growth of VSE due to excision of the IS*L3*-family element was observed 24-72 hours after exposure to vancomycin. IS*L3* insertion leads to silencing of the essential *vanHAX* gene transcription, as demonstrated by comparing Case1VSE (ΔΔCt=0,004 – 0,005) to Case1VRE (ΔΔCt=0,16 – 0,53) grown in BHI (Fig. 2). Insertion of IS*1542* upstream of *vanRS* in Case1VSE and Case1VRE leads to attenuated *vanRS* expression (ΔΔCt=0,08 – 0,20). Accordingly, the observed expression of *vanHAX* was reduced in Case1VRE relative to BM4147.

The *vanA* gene cluster was located on a transferable broad host-range plasmid also detected in outbreak isolates with different pulsotypes and in the *E. faecalis* isolate (Fig. 3).

Conclusions

IS*L3*-like element insertion mediated the silenced VanA phenotype, which could be out-selected due to IS*L3* excision events during vancomycin exposure. IS*1542* insertion upstream of *vanRS* resulted in reduced expression of *vanHAX*. Detection of the *vanA* carrying plasmid in genetic unrelated *E. faecium* as well as in an *E. faecalis* isolate, show *in vivo* horizontal transfer events. Both genotypic and phenotypic susceptibility testing is necessary to disclose these potentially vancomycin resistant isolates.

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