

Rare mechanisms of resistance in Belgian enterococci identified by WGS

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INTRODUCTION

Enterococci, particularly vancomycin-resistant *Enterococcus faecium* (VRE), are important nosocomial pathogens with limited treatment options. They can acquire high-level resistance to a broad spectrum of antibiotics. Nine types of vancomycin resistance gene clusters have been characterized. VanB positive strains are generally resistant to vancomycin and sensitive to teicoplanin.

Emergence of linezolid resistance has been limited so far. Non-susceptible organisms usually demonstrate alterations in the 23S rRNA target. A few reports have described the detection of cfr-mediated and/or optrA-mediated linezolid resistance in *E. faecalis* and *E. faecium*. Here, we report the identification of the first vanB positive teicoplanin resistant *E. faecium* strain and the first optrA positive *E. faecalis* strain in Belgium.

MATERIALS & METHODS

Species identification was performed using Malditof (Bruker). MICs for ampicillin, linezolid, teicoplanin and vancomycin were determined by e-test and interpreted according to EUCAST. Additionally, susceptibility to chloramphenicol was tested. DNA was extracted using the NucliSens EasyMAG (BioMérieux). The presence of van-genes was examined by PCR. WGS was performed using Nextera XT (2 x250bp), MiSeq (Illumina Inc.). The sequences were preprocessed by de-novo assembled (Spades v3.9.1) and annotated using RAST online server.

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RESULTS

VanB positive teicoplanin resistant *E. faecium*:

An *E. faecium* was isolated from a screening stool of a 62-year-old man with B-cell chronic lymphocytic leukemia (B-CLL) who received chemotherapy prior to allogeneic stem cell transplantation. During chemotherapy, he received multiple doses of meropenem and vancomycin due to neutropenic fever. PCR analyses of the van genes, revealed a vanB subtype, although the strain was phenotypically teicoplanin resistant (MIC > 256 µg/ml). The strain belonged to CC17, ST17. In a follow-up sample, an *E. gallinarum* was cultured also carrying the vanB gene next to vanC (Fig. 1). However, in this strain, the vanC phenotype was noticed (MIC vancomycin 8.0µg/ml, MIC teicoplanin 0.50µg/ml). WGS analysis of both strains revealed a vanB gene with a non synonymous mutation (Q124R). The *E. gallinarum* strain has a deletion in vanS_B

OptrA positive *E. faecalis*:

In another patient, an *E. faecalis* strain was isolated from a 85-year-old woman with a urinary tract infection. MIC values were 1.0, 1.0, 0.064, 16, 0.064 µg/ml, for ampicillin, vancomycin, teicoplanin, linezolid and tigecyclin respectively; the strain was resistant to chloramphenicol (MIC value 64µg/ml, CLSI). By using WGS, it was shown that the strain belonged to ST480. The suspected presence of optrA was confirmed. The optrA has 3 non-synonymous snps identified comparing to KP399637 (J Antimicrob Chemother 2015; 70: 2182–2190) cfr was not present.

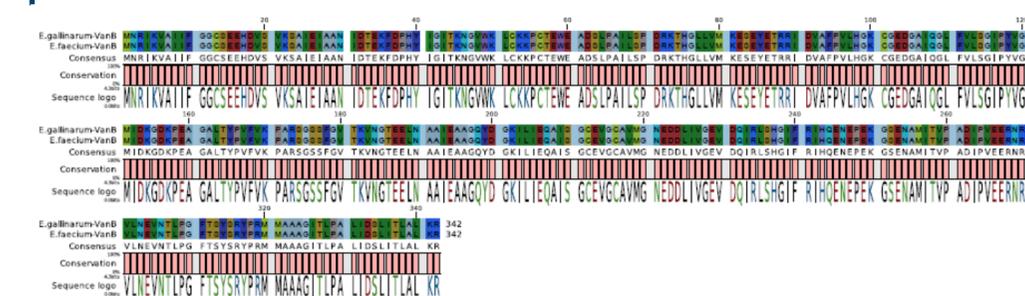


Fig 1. alignment vanB *E. faecium* and *E. gallinarum*

CONCLUSIONS

WGS was used to investigate the teicoplanin resistance in a vanB positive *E. faecium* strain. Here, the long-term treatment with vancomycin might have triggered the teicoplanin resistance. In addition, application of WGS revealed the presence of optrA as a cause of linezolid resistance in an *E. faecalis* strain.

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