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McGregor, Karen F., Young, H.-K., Nolan, C. and Woodford, N. (2001) Prevalence of the vanB2 gene cluster in VanB glycopeptide-resistant enterococci in the United Kingdom and the Republic of Ireland and its association with a Tn5382-like element. *Antimicrobial Agents and Chemotherapy*, 45 (1). pp. 367-368. ISSN 1098-6596

<http://dx.doi.org/10.1128/AAC.45.1.367-368.2001>

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## Letters to the Editor

### Prevalence of the *vanB2* Gene Cluster in VanB Glycopeptide-Resistant Enterococci in the United Kingdom and the Republic of Ireland and Its Association with a Tn5382-Like Element

The *vanB* gene cluster of enterococci confers resistance to vancomycin but not teicoplanin (4). In the United Kingdom, this cluster is found in approximately 15% of glycopeptide-resistant enterococci from hospitalized patients (8), although proportions as high as 64 and 90% have been reported in some hospitals (9, 11). Three subtypes, based on nucleotide variability, have been designated *vanB1*, *vanB2*, and *vanB3* (2, 10), respectively, and have been found within different transposons (3). We previously analyzed *vanB*-mediated resistance in isolates from Scotland, showing that 28 (88%) of 32 belonged to subtype *vanB2* (7). In this study we examined the prevalence of *vanB* subtypes among enterococci isolated in the United Kingdom and the Republic of Ireland and investigated whether *vanB* was located on a transferable element.

A total of 204 *vanB* enterococcal isolates, isolated between 1989 and 1999 from patients in 59 different hospitals in England, Wales, and Scotland and from a single hospital in the Republic of Ireland, were examined. Nucleotide sequencing and *HhaI* digestion of a fragment of the *vanB* gene was used to distinguish between the *vanB1*, *vanB2*, and *vanB3* gene clusters (7). Based on their *vanB-HhaI* restriction fragment length polymorphism (RFLP) profiles, 202 (99%) isolates contained *vanB2*. The presence of *vanB1* in two isolates was confirmed by sequence analysis.

*vanB2* has been associated with the ca. 27-kb conjugative transposon, Tn5382 (1, 3), and the closely related Tn1549 transposon (5). Therefore, we selected 28 *vanB2* isolates from the above collection (19 *Enterococcus faecium* isolates and 8 *E. faecalis* isolates from England and Wales, as well as 1 *E. casseliflavus* isolate from Dublin, Republic of Ireland), together with the 28 *vanB2 E. faecium* isolates from Scotland (7), to examine whether the *vanB2* gene cluster was associated with a similar element. *E. faecium* strain C68, previously shown to carry the Tn5382 element (1), was kindly provided by L. B. Rice for use as a positive control. Using primers specific to sequences in the left end of Tn5382 (5'-ACG CCA TGC TAT TTA CTT CCG GC-3' and 5'-GTT CTT ATT CCG CAG GTG GTG ATT-3' [1]), a 311-bp PCR fragment was generated from strain C68, and a similarly sized fragment was generated from each of the 56 selected isolates. A second set of primers (5'-TTG CAT GGT GTT CGT TGG-3' and 5'-CGG CAT CAA CGC CTT TAG-3') was used to amplify a 1,581-bp fragment containing *vanX<sub>B2</sub>* and part of the right end of Tn5382 from strain C68. A similarly sized fragment from each of the 56 isolates in this study was also amplified, suggesting that, in all cases, the *vanB2* gene cluster was associated with sequences similar to those previously seen in Tn5382.

In several VanB strains of *E. faecium* isolated in the United

States, Tn5382 contains insertion sequences and is located directly downstream of *pbp5*, which encodes a low-affinity penicillin-binding protein responsible for high-level ampicillin resistance in *E. faecium* isolates (1, 3, 6). RFLP analysis of long PCR fragments spanning *vanS<sub>B2</sub>-vanX<sub>B2</sub>* (7) from the 56 selected isolates showed that none contained ISE*nf*a200 (3) or any other additional DNA. Further PCR studies showed that Tn5382 was not located downstream of *pbp5* in any of the 47 *E. faecium* isolates.

Pulsed-field gel electrophoresis analysis of *SmaI*-digested DNA from the 56 isolates revealed 35 different types (12). Plate matings and subsequent PCR analysis of transconjugants confirmed that *vanB2* and Tn5382-like sequences were transferred from each of 3 *E. faecium* and 1 *E. faecalis* donor isolates to both *E. faecium* GE-1 and *E. faecalis* JH2-2 recipients (13). Cotransfer of vancomycin and ampicillin resistance from the *E. faecium* donors, which has been reported for isolates from the United States (1, 6), was not detected for any of the transconjugants, further confirming the lack of linkage of the *vanB2* cluster to *pbp5*.

VanB resistance among enterococci in the United Kingdom and the Republic of Ireland is dominated by the *vanB2* gene cluster and appears to have arisen by horizontal dissemination of the *vanB2* gene cluster in association with a Tn5382-type element.

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