Emergence of *vanB* *Enterococcus gallinarum* Colonization in Association with Glycopeptide Therapy[▽]

Acquired *vanA*- and *vanB*-mediated glycopeptide resistance has been well described in *Enterococcus faecalis* and *Enterococcus faecium*, and high-level *vanA*-mediated resistance has been described for *Enterococcus gallinarum* and *Enterococcus casseliflavus* (3, 5). However, reports of *vanB*-mediated resistance in *E. gallinarum* are sporadic: 2 isolates from Switzerland (6) (one patient was cocolonized with *vanB* *E. faecium*), one isolate from Australia (10), and an isolate in one poultry carcass from Sicily (7). We write to report a further case of an infection by a strain of *Enterococcus gallinarum* containing both *vanB* and *vanC1* genes in an Australian hospital.

The patient, a 67-year-old female, had undergone single-lung transplant for end-stage chronic obstructive pulmonary disease. Postoperative complications included pulmonary hemorrhage in the native lung, chylothorax, persisting infiltrates within the donor lung, and systemic inflammatory response syndrome; prolonged mechanical ventilation was required in the intensive care unit (ICU). Multiresistant methicillin-resistant *Staphylococcus aureus* (MRSA) was cultured from bronchoalveolar lavage fluid on day 10, vancomycin was commenced, and treatment continued for more than 6 weeks because tracheal aspirates and bronchial washings remained culture positive and histological sections of transbronchial biopsies showed acute epithelial inflammation. Trough vancomycin levels were maintained at >15 mg/liter for this entire period, except for 6 days when acute renal failure mandated treatment interruption.

Rectal swab screening for carriage of vancomycin-resistant enterococci (VRE) was performed 1 week posttransplant with negative results. One month after admission, repeat rectal screening for VRE resulted in the isolation of an enterococcus from an Enterococcosel agar plate (BBL, Cockeysville, MD) containing 6 mg/liter vancomycin. The isolate had typical gram morphology and was pyrrolidonyl peptidase positive. A vancomycin MIC of 32 mg/liter and teicoplanin MIC of 0.125 mg/liter were suggestive of a *vanB* genotype. The presence of a *vanB* determinant was confirmed by multiplex PCR using the LightCycler VRE detection kit (Roche Molecular Systems, Branchburg, NJ). However, the isolate was subsequently identified by Vitek 2 Compact (bioMérieux, Marcy l'Etoile, France) as *E. gallinarum*, and motility was demonstrated. To resolve the discrepancy between the phenotypic and genotypic tests, repeat identification was performed using API 20 Strep (bioMérieux, Marcy l'Etoile, France), resulting in an identification of *E. faecium*, although *E. gallinarum* was given as a possibility if vancomycin resistance had been demonstrated.

As the Roche VRE detection kit only detects *vanA*, *vanB*, and *vanB2* and -3 determinants, and to eliminate the possibility of a mixed culture, further phenotypic and genotypic tests were performed. A single colony was used both for the inoculation of VRE chromogenic media and for performance of identification and resistance gene typing by in-

house nucleic acid amplification methods (4, 8). Growth and color reactions on Chrom ID VRE (bioMérieux, Marcy l'Etoile, France) and Brilliance VRE (Oxoid, Basingstoke, United Kingdom) indicated that the isolate was a vancomycin-resistant *E. faecium* strain. However, the in-house multiplex PCRs demonstrated both a lack of *E. faecalis* and *E. faecium* genetic elements and the presence of *vanB* and *vanC1* determinants. Finally, 16S rRNA sequencing of the isolate was undertaken at a local reference laboratory (Microbiological Diagnostic Unit, University of Melbourne), showing the closest matches to strains of *E. gallinarum* and *E. casseliflavus*. The lack of pigment confirmed the isolate as a *vanB* *E. gallinarum* strain. A review of all VRE-positive rectal screens from our ICU 3 months pre- and postisolation of this *E. gallinarum* isolate failed to identify any other cases.

Previous reports have identified discrepancies between phenotypic and genotypic test results, particularly for *E. faecium* and *E. gallinarum* (6, 9). *E. gallinarum* is a relatively uncommon isolate from humans, although Australian surveillance has identified it at a higher frequency than other regions (1). It has the potential for nosocomial spread and infection outbreaks (2). Our case highlights the ability of *E. gallinarum* to acquire resistance elements in the same way as other enterococci, particularly when under antibiotic selection pressure. Speciation of enterococci in the laboratory has implications for hospital epidemiology, and confirmation of *E. gallinarum* requires multiple processes.

REFERENCES

1. Bell, J., C. Paton, and J. Turnidge. 1998. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. *J. Clin. Microbiol.* **36**:2187–2190.
2. Contreras, G. A., C. A. DíazGranados, L. Cortes, J. Reyes, S. Vanegas, D. Panesso, S. Rincón, L. Díaz, G. Prada, B. E. Murray, and C. E. Arias. 2008. Nosocomial outbreak of *Enterococcus gallinarum*: untaming of rare species of enterococci. *J. Hosp. Infect.* **70**:346–352.
3. Corso, A., D. Faccione, P. Galletti, A. Togneri, H. Lopardo, R. Melano, V. Rodríguez, M. Rodríguez, and M. Galas. 2005. First report of *vanA* *Enterococcus gallinarum* dissemination within an intensive care unit in Argentina. *Int. J. Antimicrob. Agents* **25**:51–54.
4. Dutka-Malen, S., S. Evers, and P. Courvalin. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J. Clin. Microbiol.* **33**:24–27.
5. Foglia, G., M. Del Grosso, C. Vignaroli, P. Bagnarelli, P. E. Varaldo, A. Pantosti, and F. Biavasco. 2003. Molecular analysis of Tn1546-like elements mediating high-level vancomycin resistance in *Enterococcus gallinarum*. *J. Antimicrob. Chemother.* **52**:772–775.
6. Liassine, N., R. Frei, I. Jan, and R. Auckenthaler. 1998. Characterization of glycopeptide-resistant enterococci from a Swiss hospital. *J. Clin. Microbiol.* **36**:1853–1858.
7. Mammina, C., A. M. Di Noto, A. Costa, and A. Nastasi. 2005. *VanB-VanC1 Enterococcus gallinarum*, Italy. *Emerg. Infect. Dis.* **11**:1491–1492.
8. Miele, A., M. Bandera, and B. P. Goldstein. 1995. Use of primers selective

- for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in *vanA* isolates. Antimicrob. Agents Chemother. **39**:1772–1778.
9. Ruoff, K. L., L. de la Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferraro. 1990. Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. **28**:435–437.
10. Schooneveldt, J. M., R. K. Marriott, and G. R. Nimmo. 2000. Detection of a *vanB* determinant in *Enterococcus gallinarum* in Australia. J. Clin. Microbiol. **38**:3902.

Andrew A. Mahony
Michael B. Huysmans*
Lisa Liolios
James A. Faull
Jim De Boer
Denis W. Spelman
Department of Microbiology
Alfred Hospital
Commercial Rd.
Melbourne 3004, Victoria, Australia

*Phone: 61-3-9076-2000
Fax: 61-3-9076-2227
E-mail: M.Huysmans@alfred.org.au

[†]Published ahead of print on 31 March 2010.