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CASE REPORT

Vancomycin-Variable *Enterococcus faecium*: *In Vivo* Emergence of Vancomycin Resistance in a Vancomycin-Susceptible Isolate

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We report the emergence of vancomycin resistance in a patient colonized with a *vanA*-containing, *vanRS*-negative isolate of *Enterococcus faecium* which was initially vancomycin susceptible. This is a previously undescribed mechanism of drug resistance with diagnostic and therapeutic implications.

CASE REPORT

A 69-year-old man with a medical history of type II diabetes mellitus, urinary retention, and colon adenocarcinoma with hepatic metastases causing portal hypertension and chronic ascites was admitted to the hospital with *Escherichia coli* sepsis and bleeding esophageal varices. He was known from an admission for spontaneous bacterial peritonitis 3 weeks previously to be colonized with an *Enterococcus faecium* isolate that was positive for *vanA* by PCR (Xpert *vanA/vanB* assay; Cepheid, Sunnyvale, CA) but susceptible to vancomycin (MIC = 1 μg/ml), and his admission rectal swab (obtained on day 0) yielded the same organism.

He received piperacillin-tazobactam for 2 days and then 8 days of ciprofloxacin for treatment of his bacteremia. Because of suspected recurrent sepsis on day 12, he received piperacillin-tazobactam from day 12 to 17 and intravenous vancomycin from day 12 to 14. Urine and ascitic fluid cultures yielded vancomycin-susceptible *E. faecium*, and a Tenckhoff catheter which had been inserted for control of ascites was removed. Other specimens submitted for culture were negative. On day 20, daily ceftriaxone was initiated as prophylaxis for spontaneous bacterial peritonitis. He died from bleeding esophageal varices and hepatorenal syndrome on day 29.

Screening rectal swabs obtained on days 10, 12, 22, and 24, ascitic fluid obtained on days 2 and 14, and urine obtained on day 10 all yielded *E. faecium* organisms positive for the *vanA* gene by PCR. Vancomycin MICs determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines (1) and by Etest according to the manufacturer's specifications and teicoplanin MICs determined using Etest and the Vitek2 (bioMérieux) are shown in Table 1. All isolates were susceptible to linezolid and daptomycin. There was no evidence of heteroresistance in the vancomycin-susceptible isolates. The pulsed-field gel electrophoresis patterns of all isolates were indistinguishable.

Plasmids were isolated from the vancomycin-susceptible isolate taken on day 2 (Pr0) and the vancomycin-resistant isolate taken on day 24 (Ps24) and were sequenced using a 454 sequencer (Roche, Basel, Switzerland). In both the vancomycin-susceptible and -resistant isolates, plasmids contained *vanHAXYZ* but lacked *vanRS*. Whole-genome sequencing did not reveal any chromosomal insertion of an additional Tn*1546* transposon or the presence of *vanRS* or *vanRS* homologues or another known vancomycin resistance operon (as described in the accompanying paper [2]).

Vancomycin-resistant *Enterococcus faecium* is widely disseminated (3–5). The predominant mechanism of resistance is encoded on transposon Tn*1546*, which is commonly carried on a plasmid and includes a two-component regulator gene (*vanRS*) and a gene cluster (*vanHAXYZ*) encoding the resistance mechanism (5). The *vanA* gene encodes a ligase that catalyzes the linkage of D-alanine and D-lactate, which replaces the typical D-alanine D-alanine precursor for peptidoglycan, thereby decreasing the affinity of glycopeptide antibiotics for their target site (4, 6).

Despite the tendency for insertion sequences to cause structural alterations within *vanRS* and *vanHAXYZ*, all isolates of *E. faecium* containing the Tn*1546* plasmid reported before 2011 were resistant to glycopeptide antibiotics, with only a few heteroresistant isolates being identified (7–9). Because of the correlation of *vanA* gene carriage and glycopeptide resistance, and because molecular methods offer high sensitivity and faster turnaround time, many clinical microbiology laboratories have introduced the detection of *vanA* as a surrogate marker of vancomycin resistance.

Glycopeptide-susceptible, *vanA*-bearing *E. faecium* was described for the first time when six isolates were obtained from routine patient screening samples from a single hospital in Quebec, Canada, in 2011 (10). Of the six isolates reported, one carried a modified Tn*1546* plasmid with a deletion of *vanR*, and five carried a modified Tn*1546* plasmid with deletions of *vanR* and *vanS*. Despite the presence of *vanHAXYZ*, the isolates were susceptible to vancomycin on phenotypic testing (10).

Herein, we report a novel phenotype of an apparent nonheteroresistant *E. faecium* isolate with variable resistance to vanco-

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TABLE 1 Clinical and microbiologic details of *vanA* bearing VRE isolate before and after patient exposure to vancomycin[a]

Day of admission	Specimen	Source	MIC (μg/ml)[b]	
			Vancomycin	Teicoplanin
Prior to vancomycin treatment				
0	Pr0	Rectal swab	1	1.5
2	Pr2	Ascitic fluid	1	1.5
10	Pr10	Urine	1	1.5
12	Pr12	Rectal swab	1	NT
Concurrent with vancomycin treatment (day 12–14)				
14	Co14a	Ascitic fluid	1	NT
14	Co14b	Rectal swab	1	NT
After vancomycin treatment				
22	Ps22	Rectal swab	>256	>256
24	Ps24	Rectal swab	>256	>256

[a] All isolates tested were susceptible to linezolid and quinipristin-dalfopristin and positive by PCR for *vanA*.
[b] NT, not tested.

mycin. This initially susceptible *E. faecium* isolate, which contained a modified Tn*1546* plasmid bearing *vanHAXYZ* but not *vanRS*, acquired vancomycin resistance *in vivo* following exposure to vancomycin. The potential for vancomycin resistance to arise following vancomycin exposure creates a risk of treatment failure. This situation would be complicated by the fact that traditional culture and susceptibility testing would not be able to identify the risk; both phenotypic and molecular methods are needed to detect this strain.

Several features of this isolate require further investigation. The distribution of this phenotype is not known. It has now been detected in at least 13 hospitals in Ontario and Quebec, with more than 95 patients identified as colonized or infected with *vanA*-containing vancomycin-susceptible strains of *E. faecium* in 2012 (2, 10, 11). The strain has also been shown to be readily transmitted from one patient to another in a hospital setting, with 7 Ontario hospitals reporting 1 to 42 affected patients in 2012 (10, 11). While the phenotype of vancomycin susceptibility changing may require specific host strain characteristics that have not yet been elucidated, the potential of this plasmid to pass to other species or strains clearly exists. The mechanism of resistance for these isolates is not established. Further phenotypic and genetic characterization of these strains and the modified plasmid both *in vitro* and *in vivo* is necessary to ensure that a novel *vanA*-independent resistance mechanism is not present, or to identify the genes responsible for regulating expression of the *vanHAXYZ* complex.

It is not clear whether the development of vancomycin resistance upon vancomycin exposure is a unique phenomenon in this isolate in this patient or common among isolates of this strain, nor can we be certain from this one case whether the development of vancomycin resistance was causally associated with vancomycin exposure. In light of this case, however, it seems prudent to consider therapy with antibiotics other than vancomycin when managing patients with infection due to this type of *E. faecium* until more data are available to determine whether and how frequently resistance may arise on therapy.

This report represents the first isolate of vancomycin-susceptible *Enterococcus faecium* known to become resistant to vancomycin following drug exposure. We propose the name "vancomycin-variable *Enterococcus*" (VVE) to describe such isolates. They potentially represent an important clinical and microbiologic challenge.

REFERENCES

1. **Clinical and Laboratory Standards Institute M100-S24.** 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
2. **Szakacs TA, Kalan L, McConnell MJ, Eshaghi A, Shahinas D, McGeer A, Wright GD, Low DE, Patel SM.** 2014. Emergence of vancomycin-susceptible *Enterococcus faecium* containing the wild-type *vanA* gene. J. Clin. Microbiol. **52:**1682–1686. http://dx.doi.org/10.1128/JCM.03563-13.
3. **Leclercq R, Derlot E, Duval J, Courvalin P.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:**157–161. http://dx.doi.org/10.1056/NEJM198807213190307.
4. **Uttley AH, Collins CH, Naidoo J, George RC.** 1988. Vancomycin-resistant enterococci. Lancet. **i:**57–58.
5. **Courvalin P.** 2006. Vancomycin resistance in gram-positive cocci. Clin. Infect. Dis. **42**(Suppl 1)**:**S25–S34. http://dx.doi.org/10.1086/491711.
6. **Arthur M, Reynolds PE, Depardieu F, Evers S, Dutka-Malen S, Quintiliani R, Jr, Courvalin P.** 1996. Mechanisms of glycopeptide resistance in enterococci. J. Infect. **32:**11–16. http://dx.doi.org/10.1016/S0163-4453(96)80003-X.
7. **Darini AL, Palepou MF, Woodford N.** 2000. Effects of the movement of insertion sequences on the structure of VanA glycopeptide resistance elements in *Enterococcus faecium*. Antimicrob. Agents Chemother. **44:**1362–1364. http://dx.doi.org/10.1128/AAC.44.5.1362-1364.2000.
8. **Alam MR, Donabedian S, Brown W, Gordon J, Chow JW, Zervos MJ, Hershberger E.** 2001. Heteroresistance to vancomycin in *Enterococcus faecium*. J. Clin. Microbiol. **39:**3379–3381. http://dx.doi.org/10.1128/JCM.39.9.3379-3381.2001.
9. **Khan SA, Sung K, Layton S, Nawaz MS.** 2008 Heteroresistance to vancomycin and novel point mutations in Tn*1546* of *Enterococcus faecium* ATCC 51559. Int. J. Antimicrob. Agents. **31:**27–36. http://dx.doi.org/10.1016/j.ijantimicag.2007.08.007.
10. **Gagnon S, Levesque S, Lefebvre B, Bourgault AM, Labbe AC, Roger M.** 2011. vanA-containing *Enterococcus faecium* susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn*1546*. J. Antimicrob. Chemother. **66:**2758–2762. http://dx.doi.org/10.1093/jac/dkr379.
11. **McGeer A, Fleming CA.** 2013. Antimicrobial resistance in common hospital pathogens in Ontario: report 2012. QMP-LS News. http://www.qmpls.org/KnowledgeCentre/Newsletter/CurrentIssue/tabid/88/entryid/303/Default.aspx.