Laboratory Contamination of Specimens with Quality Control Strains of Vancomycin-Resistant Enterococci in Ontario

K. C. Katz, A. McGeer, D. E. Low, and B. M. Willey*

Department of Microbiology, Toronto Medical Laboratories and Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

Received 27 February 2002/Returned for modification 29 March 2002/Accepted 29 April 2002

Cross-contamination with laboratory control strains of vancomycin-resistant *Enterococcus faecalis* was documented in 15 clinical specimens from nine clinical microbiology laboratories in Ontario, Canada. Laboratories should be alert to the possibility of contamination of specimens with vancomycin-resistant enterococci from the laboratory environment. Molecular typing of strains may assist in elucidating the source of such contamination.

Current National Committee for Clinical Laboratory Standards guidelines (10) recommend the use of *Enterococcus faecalis* ATCC 51299 for the quality control of culture media used in the detection of acquired aminoglycoside and glycopeptide resistance in enterococci. This multiply drug-resistant strain harbors the *vanB* gene cluster, whose presence results in a vancomycin MIC of 32 µg/ml and susceptibility to teicoplanin. It also harbors a bifunctional gene that confers high-level resistance to both gentamicin and streptomycin. Commercial suppliers of commonly used automated susceptibility testing systems also recommend the use of this strain for quality control of their products.

Environmental contamination of clinical microbiology laboratories with vancomycin-resistant enterococci (VRE) has been previously described. Collins and colleagues demonstrated recovery of VRE on 10% of laboratory surfaces tested (2). Our group has described laboratory surface contamination in 25% of samples taken (B. M. Willey, D. E. Low, and A. J. McGeer, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 759, 1999). To date, however, contamination of clinical specimens has not been reported.

Since VRE were first reported in Canada in the early 1990s, our laboratory has collected and characterized most of the enterococci with acquired resistance that have been identified in the province of Ontario. Because of the existence of surveillance networks and limited molecular typing resources, many clinical laboratories have also referred to our laboratory all presumptive VRE isolated from clinical or surveillance specimens for confirmation of vancomycin resistance and/or for molecular typing. Laboratories in other tertiary hospitals with typing capability have also shared with us type isolates of outbreak strains (A. Berntson, K. Green, B. M. Willey, C. A. Fleming, A. Blacklock, H. Richardson, D. E. Low, and A. McGeer, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-154, 1997).

VRE isolates were identified to the species level with a

combination of rapid and conventional biochemical tests (1, 4, 12), and their vancomycin resistance phenotypes were determined by performing broth microdilution tests of susceptibility (9) to antimicrobial agents including vancomycin, teicoplanin, and aminoglycosides. Vancomycin resistance in *E. gallinarum* and *E. casseliflavus* was considered to be intrinsic and not acquired, except in the isolates for which the vancomycin MIC was ≥ 16 µg/ml. Every confirmed VRE isolate was compared to all previously characterized clones and subclones by using *Sma*I pulsed-field gel electrophoresis (PFGE) in accordance with consensus guidelines (7, 8, 11). Each newly identified VRE clone was genotypically characterized by polymerase chain amplification of *vanA* and *vanB* resistance genes (3).

From 1993 (the year of the first identification of a VRE isolate in Ontario) to 1999, only seven clones of vancomycin-resistant *E. faecalis* were identified in Ontario. Three of these were identified in more than one patient (each of the remaining four was identified in single patients who also carried a resistant *E. faecium* strain). One of these three clones, designated PFGE strain K, was identified in six patients in hospitals in one geographic area. The epidemiology of the remaining two clones was unusual in that isolates were identified from individual patients without vancomycin-resistant *E. faecium*, no related clinical isolates of VRE were identified, screening of contacts yielded negative results, and VRE could not be identified in repeat specimens from each patient. Isolates of these two clones were identified for nine of the 121 clinical microbiology laboratories in Ontario and were from various specimens obtained from 14 patients and one staff member in 12 geographically distant health care facilities. None of these facilities had endemic or concurrent epidemic VRE, and none of the patients in question had risk factors for VRE (e.g., hospitalization in an area with known VRE, exposure to a colonized patient, or prolonged exposure to vancomycin). As in the patient cases, the hospital worker, who had no patient contact, had no known exposure to or risk factors for VRE, and repeat specimens from the worker and screening specimens from contacts were negative (Table 1).

A comparison of the PFGE DNA fingerprints generated by the 15 isolates with those of known clones from our collection indicated that all but one of those from the *vanB* isolates of *E.*

* Corresponding author. Mailing address: Department of Microbiology, Room 1460, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5. Phone: 416-586-3121. Fax: 416-586-3140. E-mail: bwilley@mtsinai.on.ca.

TABLE 1. Episodes of contamination of clinical specimens with VRE laboratory control strains

Specimen sampling date	Type of facility (facilities A to L) ^a	Type of laboratory (labs a to i) ^b	Specimen source ^c	<i>Enterococcus</i> species	Vancomycin resistance genotype	PFGE pattern of laboratory isolate	PFGE pattern of control strain in use in laboratory
06/26/96	NH (A)	PCL (a)	Urine		<i>vanB</i>	ATCC 51299	ATCC 51299
10/03/96	CH (B)	PCL (b)	Urine	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
10/03/96	CH (B)	PCL (b)	Urine	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
10/04/96	CH (C)	PCL (c)	Urine	<i>E. faecalis</i>	<i>vanA</i>	LPTP 9307-2	LPTP 9307-2
11/14/96	CH (D)	PHL (d)	Stool for <i>C. difficile</i>	<i>E. faecalis</i>	<i>vanA</i>	LPTP 9307-2	LPTP 9307-2
11/14/96	CH (E)	PHL (d)	Stool for <i>C. difficile</i>	<i>E. faecalis</i>	<i>vanA</i>	LPTP 9307-2	LPTP 9307-2
11/14/96	CH (F)	PHL (d)	Stool for <i>C. difficile</i>	<i>E. faecalis</i>	<i>vanA</i>	LPTP 9307-2	LPTP 9307-2
02/24/97	CH (B)	PHL (b)	Thigh ulcer	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
02/24/97	CH (B)	PCL (b)	Abscess	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
04/29/97	CH (G)	RHL (e)	Rectum	<i>E. faecalis</i>	<i>vanB</i>	K	K
12/09/97	TCH (H)	AHL (f)	Wound	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
02/10/98	TCH (I)	AHL (g)	Unknown	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
09/04/98	TCH (J)	AHL (h)	Rectum	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
03/12/99	LTC (K)	AHL (i)	Urine	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
03/24/99	RC (L)	AHL (i)	Urine	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299

^a NH, nursing home; CH, community hospital; TCH, tertiary-care hospital; LTC, long-term care; RC, rehabilitation center.

^b PCL, private community laboratory; PHL, public health laboratory; RHL, regional public hospital laboratory; AHL, academic hospital laboratory.

^c *C. difficile*, *Clostridium difficile*.

faecalis were indistinguishable from those from the ATCC 51299 strain. The DNA fingerprint of the other *vanB E. faecalis* isolate was indistinguishable from that of the strain previously identified in the six Ontario patients (K strain). The DNA fingerprint of the *vanA* clone was indistinguishable from that of Ontario's first VRE, an *E. faecalis* isolate. This strain, called LPTP 9307-2, had been provided to laboratories throughout the province in July of 1993 by Quality Management Program-Laboratory Services (QMP-LS). QMP-LS is the agent designated by the Ontario Ministry of Health and Long-Term Care and the Ontario Medical Association to carry out examination and evaluation of testing performance proficiency in clinical laboratories (5). As the National Committee for Clinical Laboratory Standards had not yet recommended the use of ATCC 51299 in 1993, strain LPTP 9307-2 was adopted as a control organism in a number of these laboratories. In each case, the control strain being used in the microbiology laboratory at the time of isolation of the clinical specimen matched the patient isolate (see Table 1). This included the laboratory that identified the K strain in the employee's specimen.

Review of laboratory procedures in each laboratory revealed no break in practice or other event that might have permitted cross-contamination of patient specimens with control strains. All of the laboratories routinely plated specimens in biological safety cabinets which are located away from the worktop surfaces. In three of the nine laboratories, contamination occurred on more than one occasion. In most cases, the original

specimens had already been discarded by the time the PFGE results indicated probable laboratory contamination and so were not available for reprocessing. On one occasion, however, the original swab had been placed into broth, which grew an organism that, when recultured, was indistinguishable from the ATCC control strain, according to the results of antimicrobial susceptibility profile and PFGE testing.

After two contamination events with ATCC 51299 were identified in a single laboratory (academic hospital laboratory a; see Table 1) within a 2-week period, an investigation to identify the source of laboratory environmental contamination was undertaken. A total of 28 environmental samples were obtained, with the majority being from the urine and reference benches where VRE were most frequently isolated and tested. Six of the 28 samples grew VRE (Table 2). Four of the six were identified as harboring *vanB E. faecalis*, and two were identified as harboring *vanA E. faecium*. Three of the four *E. faecalis* samples had biochemical, antibiogram, and PFGE patterns highly related to or indistinguishable from those of the ATCC 51299 VRE control strain and the urine specimens of the two patients. The ATCC 51299 strain was exclusively found on and around the urine bench where the contamination of the clinical specimens had presumably occurred and where it had been used as a quality control strain. The two *vanA E. faecium* isolates belonged to an epidemic clone (PFGE type LL) most commonly identified in Ontario and were from samples from the reference bench where the VRE isolates referred from

TABLE 2. Laboratory environmental samples that grew VRE

Specimen source	<i>Enterococcus</i> species	Vancomycin resistance genotype	PFGE pattern of laboratory isolate	PFGE pattern of control strain in use in laboratory
Computer keyboard	<i>E. faecalis</i>	<i>vanB</i>	K	ATCC 51299
Urine workstation chair back	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
Reference workstation chair back	<i>E. faecium</i>	<i>vanA</i>	LL	ATCC 51299
Urine workstation wire rack	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
Urine workstation MIC panel cover	<i>E. faecalis</i>	<i>vanB</i>	ATCC 51299	ATCC 51299
Reference workstation workbench surface	<i>E. faecium</i>	<i>vanA</i>	LL	ATCC 51299

other facilities were processed. The presence of *vanB E. faecalis* strain K at the Vitek bench workstation was confirmed. There was no obvious reason for the presence of this strain in this location.

Corrective measures to prevent further contamination of patient specimens by control strains included removal of all enterococci with acquired vancomycin resistance from quality control protocols (6) and frequent and thorough laboratory surface decontamination. Many Ontario laboratories replaced these organisms with alternative strains amenable to control in laboratory VRE media, such as *E. gallinarum* ATCC 35038 (vancomycin MIC, 8 µg per ml). Use of this strain has the advantage that it is better able to maintain quality control of vancomycin concentrations in surveillance media (6 µg of vancomycin per ml). Since the recommendation from the QMP-LS control program in 1999 of the use of this control strain, no further episodes of cross-contamination have been documented.

In conclusion, environmental laboratory contamination with VRE has led to clinical microbiology specimen contamination on multiple occasions in numerous accredited microbiology laboratories in Ontario. We have been unable to identify specific breaks in technique that would explain such contamination and hypothesize that the persistence of VRE in the environment, combined with inadvertent breaks in optimal practice, can lead to the extension of environmental contamination and, ultimately, to the contamination of clinical specimens. PFGE typing of strains may help to recognize such a problem when the presence of VRE is suspected. It is possible that in higher-prevalence institutions, laboratory cross-contamination of clinical specimens is even more likely to occur. In these settings, however, the contamination is just as likely to originate from the processing of clinical isolates (of a variety of strains), making the identification of episodes of laboratory crosscontamination more difficult.

REFERENCES

1. Chen, D. K., L. Pearce, A. McGeer, D. E. Low, and B. M. Willey. 2000. Evaluation of D-xylose and 1% methyl- α -D-glucopyranoside fermentation tests for distinguishing *Enterococcus gallinarum* from *Enterococcus faecium*. J. Clin. Microbiol. **38**:3652–3655.
2. Collins, S. M., D. M. Hacek, L. A. Degen, M. O. Wright, G. A. Noskin, and L. R. Peterson. 2001. Contamination of the clinical microbiology laboratory with vancomycin-resistant enterococci and multidrug-resistant *Enterobacteriaceae*: implications for hospital and laboratory workers. J. Clin. Microbiol. **39**:3772–3774.
3. 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. (Erratum, **33**:1434.)
4. Facklam, R. R., D. F. Sahm, and L. M. Teixeira. 1999. *Enterococcus*, p. 297–315. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Laboratory Proficiency Testing Program. 1993. Committee comments. Survey B-9307. Laboratory Proficiency Testing Program, Toronto, Ontario, Canada.
6. Laboratory Proficiency Testing Program. 1999. Committee comments. Survey B-9906. Laboratory Proficiency Testing Program, Toronto, Ontario, Canada.
7. Morrison, D., N. Woodford, S. P. Barrett, P. Sisson, and B. D. Cookson. 1999. DNA banding pattern polymorphism in vancomycin-resistant *Enterococcus faecium* and criteria for defining strains. J. Clin. Microbiol. **37**:1084–1091.
8. Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock. 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28**:2059–2063.
9. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial susceptibility testing; 9th information supplement. Document M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulse-field electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
12. Willey, B. M., R. N. Jones, A. McGeer, W. Witte, G. French, R. B. Roberts, S. G. Jenkins, H. Nadler, and D. E. Low. 1999. Practical approach to the identification of clinically relevant *Enterococcus* species. Diagn. Microbiol. Infect. Dis. **34**:165–171.