JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2006, p. 4009–4013 Vol. 44, No. 11
0095-1137/06/$08.00+0 doi:10.1128/JCM.00195-06

Molecular Epidemiology of Hospital-Acquired Vancomycin-Resistant Enterococci[▽]

Marianne Abele-Horn,[1]* Ulrich Vogel,[1] Ingo Klare,[3] Carola Konstabel,[3] Rene Trabold,[1] Risa Kurihara,[1] Wolfgang Witte,[3] Wolfgang Kreth,[2] Paul-Gerhardt Schlegel,[2] and Heike Claus[1]

Institute for Hygiene and Microbiology, University of Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Germany[1]; Department of Pediatrics, University of Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Germany[2]; and Robert Koch Institute, Wernigerode Branch, Burgstr. 37, 38855 Wernigerode, Germany[3]

Received 30 January 2006/Returned for modification 25 March 2006/Accepted 26 August 2006

Vancomycin-resistant *Enterococcus faecium* strains are a significant cause of nosocomial infections in predisposed patients. Multiple-locus variable-number tandem repeat analysis (MLVA) has been validated recently by use of a global strain collection. In this report, we applied MLVA together with multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) to type 14 isolates from three clusters of patients colonized or infected with vancomycin-resistant *Enterococcus faecium* and another 10 epidemiologically unrelated isolates from the same hospital. The clusters could be distinguished by all three typing methods, which proved to be concordant. PFGE patterns provided the highest resolution. We observed seven sequence types (ST), six MLVA types (MT), and nine distinct ST/MT combinations. The combination of MLST and MLVA may be an alternative to PFGE in hospital epidemiology, providing the benefits of high accuracy, reproducibility, and portability.

Within the last 10 years, the proportion of vancomycin-resistant *Enterococcus faecium* (VRE) strains collected at hospitals increased in the United States to 20 to 40% (22) and in some European countries to more than 10% (2002 Annual Report of the European Antimicrobial Resistance Surveillance System [www.earss.rivm.nl]). In comparison to incidences in other countries, VRE incidences in German hospitals have been low in the past (2 to 4% and in some clinics below 1% [15]). However, higher incidences, up to 25%, have been reported recently from several hospitals in southwestern Germany (14), and nosocomial outbreaks of VRE have also been reported (2, 14, 29). Widely disseminated VRE clones have been reported previously as the source of the high degree of vancomycin resistance resulting in increasing nosocomial infections in hospitals (21). It has been suggested that the virulence gene *esp* is a characteristic feature of isolates involved in nosocomial outbreaks (20, 30). As VRE infections appear to be more deadly and more costly than infections caused by vancomycin-susceptible strains (23), epidemiological data concerning occurrence and spread of these microorganisms have to be compiled, and VRE isolates have to be epidemiologically investigated.

Several molecular typing schemes have been developed to study the epidemiology of VRE. Of these, pulsed-field gel electrophoresis (PFGE) of genomic restriction fragments has been considered the gold standard because of its high degree of isolate differentiation (18). However, PFGE is hampered by a variety of disadvantages: (i) the method is time-consuming, (ii) comparability between laboratories is unsatisfying, and thus international databases can be compiled only by applying rigorous quality assurance and quality control, as in the case of PulseNet (25), and (iii) banding patterns are only partially informative with regard to phylogenetic relationships. Multilocus sequence typing (MLST) and multiple-locus variable-number tandem repeat analysis (MLVA) have been developed recently to recognize genetically related and potential epidemic isolates of *E. faecium* (11, 27). MLST was recommended for strain characterization and long-term epidemiological investigations. It was shown that few clones emerged recently carrying the vancomycin resistance determinant. MLST confirmed the unrelatedness of human and nonhuman VRE, as had been established by PFGE beforehand, and confirmed subgroup C1 as responsible for human cases (1). Several authors used MLST for outbreak investigations thereafter (17, 24, 29). The clonal complex of sequence type 17 (ST-17) was repeatedly shown to be related to the VRE problem worldwide.

Despite the high accuracy and portability of MLST (28), attempts have been made in molecular epidemiology to reduce the cost and labor required for MLST for several PCRs and DNA sequencing reactions. MLVA has become popular in this respect for a variety of organisms, e.g., those related to bioterrorism (7, 13, 16). The MLVA scheme for *E. faecium* developed by Top et al. (27) is especially easy to handle, because the repeats selected are of a size which can be distinguished by visual inspection using standard agarose gel electrophoresis. Furthermore, a user-friendly Internet-based database can be interrogated to assign MLVA types. MLVA has been shown to be concordant with MLST (27). A different MLVA scheme has been described for *E. faecalis* (26).

In the present study, we investigated VRE from three clusters at the University Hospital of Würzburg by MLST and MLVA and compared these data to PFGE data.

* Corresponding author. Mailing address: Institute for Hygiene and Microbiology, University of Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Germany. Phone: 49-931-201-46941. Fax: 49-931-201-46445. E-mail: mhorn@hygiene.uni-wuerzburg.de.

▽ Published ahead of print on 27 September 2006.

MATERIALS AND METHODS

Bacterial isolates. *E. faecium* was identified by VITEK 2 (bioMérieux, Nürtingen, Germany). Vancomycin and teicoplanin susceptibilities were assessed by the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (4). Isolates with a vancomycin MIC of ≥2 μg/ml were analyzed by PCR for the presence of the *vanA* and *vanB* genes, respectively, as described earlier (5). Fourteen VRE isolates with an epidemiological link were obtained in 2001, 2002, and 2004 from patients of the University Hospital of Würzburg (Table 1). Outbreaks of VRE were identified by laboratory surveillance and by regular visits of hospital wards by the infection control nurse and the clinical microbiologist in charge. Clinical epidemiological assessment was made by informal oral inquiries of the hospital staff. For determination of the discriminatory power of MLVA, another 10 VRE strains, which were obtained between 2000 and 2004 from patients of the University Hospital, were sampled at random from the strain collection of the Institute for Hygiene and Microbiology. The institute provides bacteriological service to the University Hospital of Würzburg. This hospital is the largest referral center in the southern German region of Lower Franconia, with a population size of about 1,300,000. Only independent isolates were considered for this purpose. Independence of isolates required that the isolates were obtained from distinct patients who were taken care of at different wards of the hospital. Isolates from the same ward or the same patient were accepted only if they exhibited a different MLVA type.

MLST and MLVA. MLST was performed as described by Homan et al. (11). Sequence types were assigned using http://efaecium.mlst.net/. MLVA was performed as described recently (27). MLVA types were assigned using http://www.mlva.umcutrecht.nl. eBURST v2, available at http://eburst.mlst.net/, was used to analyze MLST and MLVA data (8). Simpson's index of discrimination was determined according to methods of Hunter and Aston (12) and Grundmann et al. (9).

PFGE, amplification of the *esp* and *hyl* genes, and antimicrobial susceptibility testing. Pulsed-field gel electrophoresis (SmaI), PCR amplification of the *esp* and *hyl* genes, and antimicrobial susceptibility testing by broth microdilution were performed at the Wernigerode branch of the Robert Koch Institute as described recently by Klare et al. (14). Similarities of PFGE patterns were estimated by a band-based similarity coefficient (Dice) according to the method of Claus et al. (3).

RESULTS AND DISCUSSION

Clusters of VRE in hospitalized patients. Our investigations revealed two clusters with *vanA*-positive *E. faecium* strains in the Department of Pediatrics. Furthermore, an additional cluster with *vanA*-positive *E. faecium* strains, which occurred in the internal oncology and orthopedics units, is described below.

The first cluster was observed at the pediatric oncology unit of the University Hospital, where VRE isolates were found in two patients with bacteremia and five asymptomatic, colonized patients between January 2001 and April 2002. The colonized patients were identified by intensified screening following the incidences of invasive VRE disease. The introduced infection control measures included weekly surveillance for VRE colonization, education of the staff, and change of antibiotic therapy regimens from imipenem as first-line treatment to piperacillin-tazobactam. Under this regimen, no further VRE isolates were identified since May 2002.

The second cluster occurred at the pediatric neonatology unit of the University Hospital. The index patient was a 6-month-old girl with a colon abnormality who underwent numerous surgical interventions and treatments with broad-spectrum antimicrobial agents. VRE was isolated from a routinely performed intraoperative swab from her colon in October 2001. Stool samples from the other patients were immediately obtained and revealed another two patients who were colonized with VRE in the time period from October 2001 until April 2002. Since May 2002, no further VRE strains were isolated.

The third cluster of VRE was detected at the internal oncology unit in spring 2004. In February 2004, stool samples of two patients screened positive for VRE. In March 2004, stool samples from another patient who suffered from *Clostridium difficile*-associated diarrhea were routinely analyzed and revealed VRE. The fourth patient (patient 19) associated with this cluster was admitted to the orthopedics unit in November 2003 and was taken care of there until March 2004. During this time, VRE was not detected in any of his stool samples. In March 2004, the patient was transferred to the internal oncology unit for treatment of anemia for 1 week. After his return to the orthopedics unit, his stool samples were positive for VRE and 2 weeks later he developed osteomyelitis with VRE. VRE typing by MLST and MLVA revealed ST-78 and MLVA type 12 (MT-12). This finding retrospectively elucidated the coincidence of VRE acquisition and a stay at the internal oncology unit, at which the same VRE clone circulated (cluster c).

Typing of VRE. Table 1 and Fig. 1 summarize the epidemiological data, microbiological findings, and typing results of VRE isolates obtained from patients involved in clusters a to c and 10 independent isolates. All isolates were positive for *vanA* and negative for *vanB* as evidenced by PCR. Despite this finding, 15 isolates were sensitive or intermediate with regard to susceptibility to teicoplanin, which is a pattern usually found with *vanB*-positive strains. This discrepancy might be the result of mutations in the *vanS* regulatory gene of *vanA*-positive isolates (6, 10, 19). The seven strains from the pediatric oncology unit were ST-17 or ST-233 and displayed MT-1, whereas the strains isolated from patients of the pediatric neonatology unit were ST-18 and MT-8. These results indicated that the strains retrieved from the two pediatric units were different clones. It is unclear whether two different clones occurred at the pediatric oncology unit or whether ST-17 and ST-233 were direct descendants from each other because they differed only in a single nucleotide polymorphism in the *pstS* gene. The strains from the internal oncology unit were ST-78 and MT-12. All identified STs belonged to clonal complex 17. ST-233 and ST-78 are single locus variants of ST-17, whereas ST-18 is a double locus variant of ST-17. One isolate from cluster b (patient 23) was positive for the *esp* gene and differed slightly in PFGE pattern from those of the two other isolates. The epidemiological link thus could not be fully confirmed. Interestingly, this isolate was retrieved 5 and 6 months after isolation of the two other isolates from this cluster, respectively. In contrast to results for cluster b, *esp* and PFGE data were consistent for the other two clusters.

MLVA, MLST, and PFGE were performed on the strains mentioned above and 10 independent isolates. PFGE patterns were largely consistent with MLVA and MLST results but, as expected, displayed a higher discrimination between strains. Whereas all strains from cluster c were identical, strains from clusters a and b in the pediatric clinic showed some band variation, as reflected by the dendrogram in Fig. 1. Interestingly, *esp*-positive strains with ST-78 and MT-12 (patients 13, 14, and 15) were found outside cluster c. In this case, PFGE provided additional informative resolution to distinguish these strains from the epidemiologically linked cases. The same holds true for two strains with ST-17/MT-1 and ST-18/MT-8 which occurred in patients unrelated to

TABLE 1. VRE strain collection analyzed in this study

Patient no.[a]	Strain no.	Date of isolation	Origin	PFGE cluster	ST	MT	Presence (+) or absence (−) of:				Result of antibiotic susceptibility testing[b]									
							esp	*hyl*	*vanA*	*vanB*	AMP	CIP	ERY	GEN[c]	LZD	MXF	Q-D	TEC[d]	OTE	VAN[d]
1*	5139/01	October 2001	Pediatric oncology unit	a	17	1	−	−	+	−	R	I	R	R (l)	S	I	S	S (4)	S	R (32)
2*	4596/01	September 2001	Pediatric oncology unit	a	233	1	−	−	+	−	R	R	R	R (l)	S	I	S	S (2)	S	R (32)
3*	194/01	January 2001	Pediatric oncology unit	a	17	1	−	−	+	−	R	R	R	R (l)	S	I	S	S (4)	S	R (64)
4*	5117/01	October 2001	Pediatric oncology unit	a	17	1	−	−	+	−	R	R	R	R (l)	S	I	S	S (4)	S	R (64)
5*	5212/01	October 2001	Pediatric oncology unit	a	233	1	−	−	+	−	R	I	R	R (l)	S	I	S	I (8)	S	R (64)
6*	1417/02	April 2002	Pediatric oncology unit	a	17	1	−	−	+	−	R	R	R	R (l)	S	R	S	S (4)	S	R (64)
7*	204/02	January 2002	Pediatric oncology unit	a	17	1	−	−	+	−	R	R	R	I	S	R	S	S (4)	S	R (64)
8	1889/01	May 2001	Internal medicine unit		17	1	+	−	+	−	R	R	R	R (l)	S	R	S	R (16)	S	R (128)
9	10218/03	June 2003	Pediatric neonatology unit		18	8	−	−	+	−	R	R	R	R (l)	S	R	S	I (8)	S	R (64)
10	4588/03	October 2003	Nephrology unit		65	5	+	−	+	−	R	R	R	R (h)	S	R	S	S (2)	S	R (32)
11	18745/02	November 2002	Surgery unit		17	16	+	−	+	−	R	R	R	R (l)	S	R	S	S (4)	R	R (16)
12	1015/04	March 2004	Nephrology unit		306	5	+	−	+	−	R	R	R	R (h)	S	R	S	R (16)	S	R (256)
13	1925/03	April 2003	Internal oncology unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (16)	S	R (128)
14	5743/03	December 2003	Urology unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	I (8)	S	R (128)
15	2177/03	February 2003	Pediatric internal unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (16)	S	R (128)
16†	4466/04	April 2004	Internal oncology unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (32)	S	R (256)
17†	678/04	March 2004	Internal oncology unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (16)	R	R (128)
18†	4181/04	March 2004	Internal oncology unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (32)	S	R (256)
19†	5127/04	April 2004	Orthopedics unit	c	78	12	+	−	+	−	R	R	R	R (h)	S	R	S	R (32)	S	R (256)
20	16699/00	October 2000	Neurosurgery unit		6	89	−	−	+	−	S	I	R	R (l)	S	S	I	I (8)	R	R (256)
21§	5110/01	October 2001	Pediatric neonatology unit	b	18	8	−	−	+	−	R	R	R	R (l)	S	R	S	S (2)	S	R (64)
22§	5335/01	November 2001	Pediatric neonatology unit	b	18	8	−	−	+	−	R	R	R	R (l)	S	R	S	S (4)	S	R (32)
23§	1394/02	April 2002	Pediatric neonatology unit		18	8	+	−	+	−	R	R	R	R (l)	S	R	S	S (4)	S	R (32)
24	1534/03	February 2003	Internal unit		18	1	+	+	+	−	R	R	R	I	S	R	S	R (64)	R	R (512)

[a] Epidemiologically observed clusters of VRE infection and carriage are indicated by symbols after the patient numbers, with each symbol indicating a separate cluster.

[b] AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; LZD, linezolid; MXF, moxifloxacin; Q-D, quinupristin-dalfopristin; TEC, teicoplanin; OTE, oxytetracycline; VAN, vancomycin; S, sensitive; I, intermediate; R, resistant.

[c] R (l), low-level resistance (≥8 μg/ml); R (h), high-level resistance (≥1,024 μg/ml).

[d] Values in parentheses given with TEC and VAN results are MICs (μg/ml).

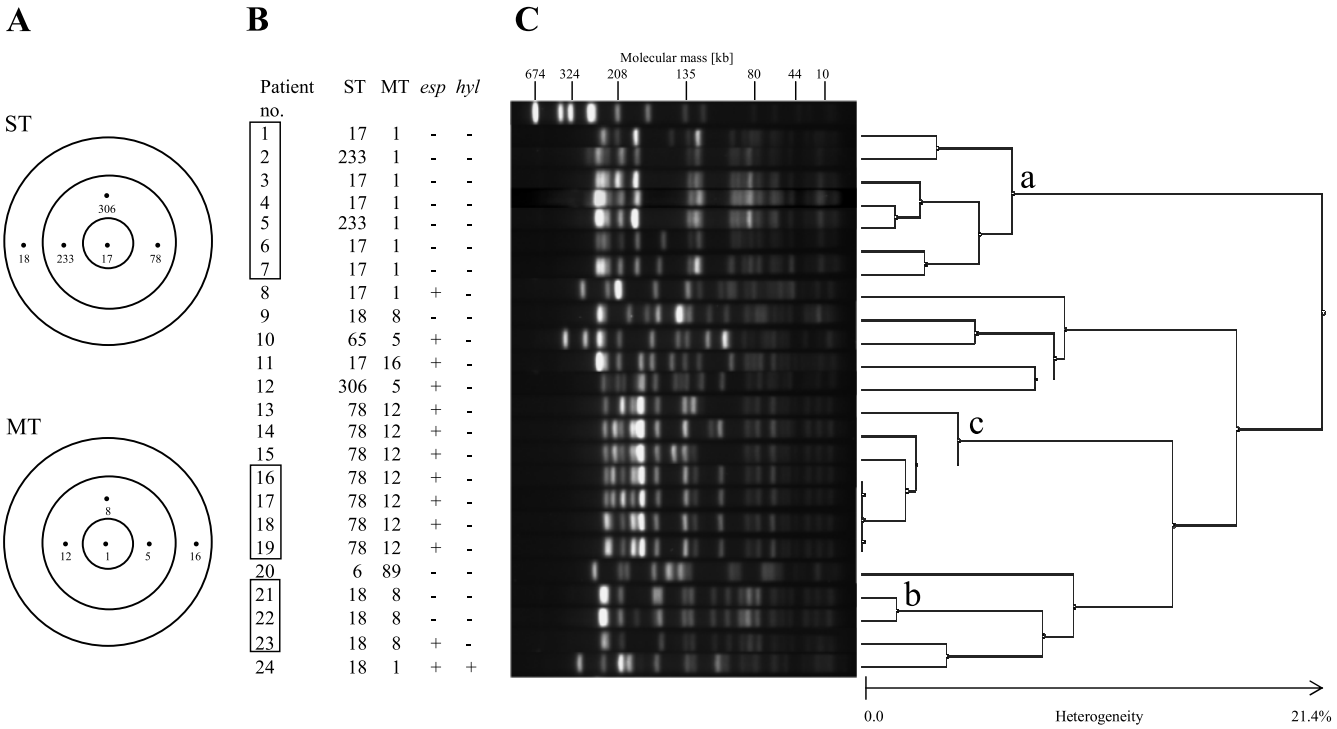

FIG. 1. Results of typing of VRE. (A) Relationships of MLST and MLVA types recovered at the University Hospital of Würzburg within a 5-year period. The founder genotype is located in the center. Single locus and double locus variants are placed in the second and third circles, respectively. (B) Patient numbers (boxes indicate epidemiologically observed clusters of VRE) and results of ST, MT, *esp*, and *hyl* analyses. +, positive; −, negative. (C) PFGE gel and dendrogram.

clusters a and b, respectively, and which exhibited distinct PFGE patterns. These data indicate that identical ST/MT patterns rarely can be observed for unrelated isolates but that additional PFGE and *esp* analysis might allow distinction of the strains.

In all strains, we found seven distinct STs, six distinct MTs, and nine distinct combinations of ST and MT, suggesting that combination of MLVA and MLST provides additional resolution. Simpson's index of diversity achieved by a combination of MLST and MLVA was determined by analysis of 14 independent isolates. The selection comprised the 10 epidemiologically unrelated isolates mentioned above, two isolates from cluster a (ST-17/MT-1 and ST-233/MT-1), and one isolate of clusters b and c. The index was fairly high, 0.912 (95% confidence interval, 0.817 to 1.000). This finding supports the hypothesis that combining MLVA and MLST may increase discrimination in comparison to each method alone. However, further analyses are needed to substantiate this finding. We would further like to highlight the impact of the *esp* PCR, which provided important information to discriminate two isolates.

To the best of our knowledge, this paper is the first MLVA application in local hospital epidemiology of VRE, which furthermore comprises MLST, PFGE, and determination of the presence of the *esp* gene. MTs were largely congruent with STs. MLVA proved to be a robust, simple, cheap, and rapid technique, but the discriminatory power of MLVA in strains recovered from patients at the University Hospital (Simpson's index of diversity, 0.846; 95% confidence interval, 0.755 to 0.937) was lower than what has been shown previously for an international strain collection (27). A possible explanation for this low index compared with that of the international collection might be sampling difference and geographic uniformity of our strains. All but one isolate (MT-89) could be assigned to a single MLVA clonal complex. This one exceptional isolate differed considerably from all others and shared only one MLVA allele with the founder type MT-1 (Fig. 1A). MLST confirmed the limited diversity of VRE strains. Our results show that VRE typing has to be interpreted carefully on the basis of diversity indices, but the important inference that can be made in many cases is the rejection of the hypothesis of identity of strains. We suggest that the combination of MLVA and MLST (and *esp* PCR) further improves resolution, meanwhile guaranteeing more-timely information than PFGE, if appropriate facilities are available.

ACKNOWLEDGMENTS

Matthias Frosch is thanked for helpful discussions and support. This publication made use of the MLST website (http://efaecium.mlst.net/), which was developed in the laboratory of Rob Willems in The National Institute of Public Health and the Environment (RIVM), The Netherlands, in collaboration with David Tribe at The University of Melbourne, Australia. We furthermore made use of the MLVA website at http://www.mlva.umcutrecht.nl (27).

REFERENCES

1. **Bonora, M. G., M. Ligozzi, M. de Fatima, L. Bragagnolo, A. Goglio, G. C. Guazzotti, and R. Fontana.** 2004. Vancomycin-resistant *Enterococcus faecium* isolates causing hospital outbreaks in northern Italy belong to the multilocus sequence typing C1 lineage. Microb. Drug Resist. **10:**114–123.
2. **Borgmann, S., D. M. Niklas, I. Klare, L. T. Zabel, P. Buchenau, I. Auten-**

rieth, and P. Heeg. 2004. Two episodes of vancomycin-resistant *Enterococcus faecium* outbreaks caused by two genetically different clones in a newborn intensive care unit. Int. J. Hyg. Environ. Health **207:**386–389.
3. **Claus, H., C. Cuny, B. Pasemann, and W. Witte.** 1998. A database system for fragment patterns of genomic DNA of *Staphylococcus aureus*. Zentbl. Bakteriol. **287:**105–116.
4. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. M100-S15. Clinical and Laboratory Standards Institute, Wayne, Pa.
5. **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.
6. **Eom, J. S., I. S. Hwang, B. Y. Hwang, J. G. Lee, Y. J. Lee, H. J. Cheong, Y. H. Park, S. C. Park, and W. J. Kim.** 2004. Emergence of *vanA* genotype vancomycin-resistant enterococci with low or moderate levels of teicoplanin resistance in Korea. J. Clin. Microbiol. **42:**1785–1786.
7. **Farlow, J., K. L. Smith, J. Wong, M. Abrams, M. Lytle, and P. Keim.** 2001. *Francisella tularensis* strain typing using multiple-locus, variable-number tandem repeat analysis. J. Clin. Microbiol. **39:**3186–3192.
8. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBurst: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:** 1518–1530.
9. **Grundmann, H., S. Hori, and G. Tanner.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192.
10. **Hashimoto, Y., K. Tanimoto, Y. Ozawa, T. Murata, and Y. Ike.** 2000. Amino acid substitutions in the VanS sensor of the VanA-type vancomycin-resistant enterococcus strains result in high-level vancomycin resistance and low-level teicoplanin resistance. FEMS Microbiol. Lett. **185:**247–254.
11. **Homan, W. L., D. Tribe, S. Poznanski, M. Li, G. Hogg, E. Spalburg, J. D. A. van Emden, and R. J. L. Willems.** 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. J. Clin. Microbiol. **40:**1963–1971.
12. **Hunter, P. R., and M. A. Aston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
13. **Keim, P., L. B. Price, A. M. Klevytska, K. L. Smith, J. M. Schupp, R. Okinaka, P. J. Jackson, and M. E. Hugh-Jones.** 2000. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J. Bacteriol. **182:**2928–2936.
14. **Klare, I., C. Konstabel, S. Müller-Bertling, G. Werner, B. Strommenger, C. Kettlitz, S. Borgmann, B. Schulte, D. Jonas, A. Serr, A. M. Fahr, U. Eigner, and W. Witte.** 2005. Spread of ampicillin/vancomycin-resistant *Enterococcus faecium* of the epidemic-virulent clonal complex-17 carrying the genes *esp* and *hyl* in German hospitals. Eur. J. Clin. Microbiol. Infect. Dis. **24:**818–825.
15. **Klare, I., C. Konstabel, D. Badstübner, G. Werner, and W. Witte.** 2003. Occurrence and spread of antibiotic resistance in *Enterococcus faecium*. Int. J. Food Microbiol. **88:**269–290.
16. **Klevytska, A. M., L. B. Price, J. M. Schupp, P. L. Worsham, J. Wong, and P. Keim.** 2001. Identification and characterization of variable-number tandem repeats in the *Yersinia pestis* genome. J. Clin. Microbiol. **39:**3179–3185.
17. **Ko, K. S., J. Y. Baek, J. Y. Lee, W. S. Oh, K. R. Peck, N. Y. Lee, W. G. Lee, K. Lee, and J. H. Song.** 2005. Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolates from Korea. J. Clin. Microbiol. **43:** 2303–2306.
18. **Kühn, I., L. G. Burman, S. Haeggman, K. Tullus, and B. E. Murray.** 1995. Biochemical fingerprinting compared with ribotyping and pulsed-field gel electrophoresis of DNA for epidemiological typing of enterococci. J. Clin. Microbiol. **33:**2812–2817.
19. **Lauderdale, T. L., L. C. McDonald, Y. R. Shiau, P. C. Chen, H. Y. Wang, J. F. Lai, and M. Ho.** 2002. Vancomycin-resistant enterococci from humans and retail chickens in Taiwan with unique VanB phenotype-*vanA* genotype incongruence. Antimicrob. Agents Chemother. **46:**525–527.
20. **Leavis, H. L., R. J. Willems, E. Top, E. M. Spalburg, A. Mascini, C. Fluit, A. Hoepelman, A. J. de Neeling, and M. J. Bonten.** 2003. Epidemic and nonepidemic multidrug-resistant *Enterococcus faecium*. Emerg. Infect. Dis. **9:**1108–1115.
21. **Murray, B. E.** 2000. Vancomycin-resistant enterococcal infections. N. Engl. J. Med. **342:**710–721.
22. **National Nosocomial Infections Surveillance System.** 1998. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from October 1986-April 1998, issued June 1998. [Online.] http:www.cdc.gov /ncidod/hip/NNIS/sar98net.pdf. Accessed May 2001.
23. **Salgado, C. D., and B. M. Farr.** 2003. Outcomes associated with vancomycin-resistant enterococci: a meta-analysis. Infect. Control Hosp. Epidemiol. **24:** 690–698.
24. **Stampone, L., M. Del Grosso, D. Boccia, and A. Pantosti.** 2005. Clonal spread of a vancomycin-resistant *Enterococcus faecium* strain among bloodstream-infecting isolates in Italy. J. Clin. Microbiol. **43:**1575–1580.
25. **Swaminathan, B., T. J. Barrett, S. B. Hunter, and R. V. Tauxe.** 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance—United States. Emerg. Infect. Dis. **7:**382–389.
26. **Titze-de-Almeida, R., R. J. L. Willems, J. Top, I. P. Rodrigues, R. F. Ferreira, H. Boelens, M. C. C. Brandileone, R. C. Zanella, M. S. S. Felipe, and A. van Belkum.** 2004. Multilocus variable-number tandem repeat polymorphism among Brazilian *Enterococcus faecalis* strains. J. Clin. Microbiol. **42:**4879–4881.
27. **Top, J., L. M. Schouls, M. J. M. Bonten, and R. J. L. Willems.** 2004. Multiple-locus variable-number tandem repeat analysis, a novel typing scheme to study the genetic relatedness and epidemiology of *Enterococcus faecium* isolates. J. Clin. Microbiol. **42:**4503–4511.
28. **Urwin, R., and M. C. Maiden.** 2003. Multi-locus sequence typing: a tool for a global epidemiology. Trends Microbiol. **11:**479–487.
29. **Werner, G., I. Klare, F.-B. Spencker, and W. Witte.** 2003. Intra-hospital dissemination of quinupristin/dalfopristin- and vancomycin-resistant *Enterococcus faecium* in a paediatric ward of a German hospital. J. Antimicrob. Chemother. **52:**113–115.
30. **Willems, R. J. L., W. Homan, J. Top, M. van Santen-Verheuvel, D. Tribe, and X. Manzioros.** 2001. Variant *esp* gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. Lancet **357:**853–855.