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Comparison of four methods, including semi-automated rep-PCR, for the typing of vancomycin-resistant *Enterococcus faecium*

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A B S T R A C T

We have assessed the performance of semi-automated rep-PCR (Diversilab®) and multilocus sequence typing (MLST) in comparison to pulsed-field gel electrophoresis (PFGE) for typing a collection of 29 epidemiologically characterized vancomycin-resistant *Enterococcus faecium* (VRE). Sixteen strains that harbored the Tn*1546* element were typed by PCR mapping. The discriminative power of the typing methods was calculated by the Simpson's index of diversity, and the concordance between methods was evaluated by the Kendall's coefficient of concordance. Semi-automated rep-PCR appeared as discriminative as PFGE and was further compared with PFGE for typing 67 VRE isolated during a hospital outbreak. Rep-PCR appeared to be more discriminative than PFGE for this second set of strains. Reproducibility of DiversiLab® was also tested against 35 selected isolates. Only three showed less than 97% similarity, indicating high reproducibility at this level of discrimination. In conclusion, semi-automated rep-PCR is a useful tool for rapid screening of VRE isolates during an outbreak, although cost of the system may be limiting for routine implementation. PFGE, which remains the reference method, should be used for confirmation and evaluation of the genetic relatedness of epidemic isolates.

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1. Introduction

Hospital-acquired infections due to bacteria that are resistant to multiply antibiotics have led to a worrying situation in many parts of the world. Among the responsible pathogens, enterococci have emerged as an increasingly important cause of nosocomial infections in the last decade, being now the fourth to fifth most prevalent nosocomial pathogen worldwide (Rice, 2001). Although these microorganisms are considered low-virulence pathogens, they may be responsible for a large variety of community- and hospital-acquired infections, such as endocarditis, bacteraemia, meningitis, wound, and urinary tract infections, and are associated with intra-abdominal infections. Among enterococci, strains belonging to the species *Enterococcus faecium* have shown increasing resistance to many antimicrobial agents, including penicillins, aminoglycosides (high-level resistance), and glycopeptides (vancomycin-resistant enterococci, VRE), thus limiting therapeutic options (Jett et al., 1994; Sava et al., 2010). Two major types of resistance to vancomycin have been described in *E. faecium*: VanA type, with high-level cross-resistance to vancomycin and teicoplanin, and VanB type, with resistance to vancomycin only. VanA-type isolates are predominant worldwide and contain transposons related to the 10.8-kb Tn*1546* element initially reported in *E. faecium* BM4147 (Dutka-Malen et al., 1990). The *vanA* gene cluster borne by these transposons includes 7 genes, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* cooperating for expression of resistance and *vanR* and *vanS* encoding a two-component regulatory system.

The control of nosocomial infections is based, in part, on tracking the spread of isolates potentially responsible for outbreaks. Due to its high discriminatory power, pulsed-field gel electrophoresis (PFGE) is considered as the reference method for molecular typing of enterococci. However, this technique has major limitations: it is time-consuming, labor-intensive, technically variable, which affects reproducibility and provides subjective-interpretable results.

By contrast, multilocus sequence typing (MLST) is a reproducible method that provides objective and unambiguous sequence types (ST). On the basis of the phylogenetic analysis of STs (Homan et al., 2002), it has been shown that increased circulation of VRE within and between hospitals was primarily due to enhanced prevalence of a distinct subpopulation consisting of a hospital-adapted *E. faecium* clonal complex (CC), designated CC17 (Willems et al., 2005). However, this technique has a lower discriminatory power than that of PFGE and is also costly and time-consuming. Overall, it is not suitable for routine analysis of outbreak isolates.

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Repetitive sequence-based PCR (rep-PCR) uses primers that target non-coding repetitive sequences interspersed in bacterial genomes (Versalovic et al., 1991). The amplified DNA fragments of different sizes can be separated by agarose gel electrophoresis, thus providing a genomic fingerprinting that allows discrimination between isolates. However, this technique initially appeared to be poorly reproducible (Malathum et al., 1998), which limited the interlaboratory comparisons. The recently commercialized DiversiLab® system (bioMérieux, Marcy l'Etoile, France) is a rep-PCR technique presenting several noticeable advantages, such as semi-automation, standardization, and higher reproducibility (Pounder et al., 2006).

Various other typing methods have been developed, such as MLVA (Top et al., 2004) and AFLP (Pangallo et al., 2008). Specifically for VanA-type VRE, insertion of insertion sequences (IS) at various loci within Tn*1546* leads to heterogeneity that allows typing (Miele et al., 1995).

The aim of this study was to evaluate the performance of some typing methods, including semi-automated rep-PCR (Diversilab®) and MLST in comparison with PFGE against a collection of 29 epidemiologically characterized VRE clinical strains. Sixteen of these strains that harbored Tn*1546* were also typed by transposon mapping. Finally, since semi-automated rep-PCR appeared as discriminative as PFGE, we evaluated this technique in comparison with PFGE on 67 VRE isolated during a hospital outbreak.

2. Materials and methods

2.1. Bacterial isolates

Two sets of vancomycin-resistant *E. faecium* were selected. First, 29 strains with various STs and PFGE profiles isolated in 15 hospitals were selected from the collection of the National Reference Centre for Enterococci (NRC-E), including 7 (24%) and 22 (76%) *E. faecium* harboring the *vanB* and *vanA* genes, respectively. The strains were typed by three techniques: PFGE, rep-PCR, and MLST. Sixteen of the VanA type isolates were also typed by transposon mapping.

The second set included 67 *vanA*-positive *E. faecium* recovered from an outbreak in a single hospital that occurred in 2008. The strains were analyzed by PFGE and rep-PCR. Thirty-five of these isolates were randomly selected for testing the reproducibility of the DiversiLab® method by having them analyzed twice by two different technicians.

2.2. Pulsed-field gel electrophoresis

PFGE was performed as described (Miranda et al., 1991). Briefly, agarose plugs containing genomic DNA were digested with SmaI (Amersham Biosciences, Orsay, France) according to the supplier's recommendations. Electrophoresis was performed with a CHEF-DRIII apparatus (Bio-Rad, Marnes-la-Coquette, France) by using the *Enterococcus* program (ramped pulse times of 5 s and 35 s at 200 V for 21 h). The PFGE patterns were analyzed by using the Fingerprinting® II software (Bio-Rad). Calculation of similarity matrices and dendrograms was obtained by using the unweighted pair group method using arithmetic averages (UPGMA). Similarity coefficients were calculated according to the method of Dice (1945). Patterns were considered as closely related or indistinguishable, if similarity was ≥95%.

2.3. MLST analysis

MLST was, as previously described (Homan et al., 2002), based on seven housekeeping genes (*atpA*, *ddl*, *gdh*, *purk*, *gyd*, *pstS*, and *adk*). Different sequences were assigned allele numbers, and different allelic profiles were assigned STs based on the *E. faecium* MLST database (http://efaecium.mlst.net).

2.4. Rep-PCR

DNA was extracted from *Enterococcus* colonies using UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA), as recommended. The DNA concentration was adjusted to 25–50 ng/μl using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). DNA amplification was performed using the DiversiLab® *Enterococcus* DNA Fingerprinting Kit (Bacterial Barcodes), according to the manufacturer's instructions, as described previously (Pounder et al., 2006). Briefly, approximately 50 ng of genomic DNA was added to 0.5 μl (2.5 U) of AmpliTaq polymerase, 2 μl kit supplied primer mix, 2.5 μl GeneAmp 10× PCR Buffer (Applied Biosystems, Foster City, CA), and 18 μl kit-supplied rep-PCR mix (MM1). PCR was performed using a Mastercycler Gradient™ (Eppendorf, Foster City, CA) under the following thermocycler conditions: initial denaturation of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 70 °C for 90 s, and a final extension of 70 °C for 3 min. The rep-PCR amplicons were separated using the chip-based LabChip® technology (Caliper Technologies Corp., Mountain View, CA) and analyzed using the web-based DiversiLab® software (version 3.4), which uses the Pearson correlation coefficient to calculate pairwise similarities between all the samples tested. The discriminant threshold was calculated by the software.

2.5. Tn1546 mapping

DNA extraction and PCR amplification were performed as previously described (Arthur et al., 1993; Dutka-Malen et al., 1990) on a subset of 16 *vanA*-positive *E. faecium* from the 29 strain collection. Overlapping DNA fragments internal to the transposon Tn*1546* were amplified with specific primer pairs (Table 1). The size of the PCR products was compared with that obtained with prototype Tn*1546* from *E. faecium* BM4147 (Dutka-Malen et al., 1990). Sequence of the PCR products that differed from the prototype was determined.

2.6. Statistical analysis

2.6.1. Discriminatory power

The discriminatory power of each typing method was assessed by calculating the Simpson's index of diversity (D) that represents the ability of a typing method to distinguish between unrelated strains (Hunter and Gaston, 1988). The index is determined by the number of types defined by the typing method and the relative frequencies of these types.

Table 1
Primers used for Tn*1546* mapping.

Primer	Sequence (5′–>3′)	Position	Size of PCR product (bp)[a]
P1	GGA-TTT-ACA-ACG-CTA-AC	22–38	P1–P2: 1308
P2	GCC-TTT-ATC-AGA-TGC-TA	1330–1314	
P3	GGT-TTT-CGA-TTA-TTG-GA	1222–1238	P3–P4: 1131
P4	AAA-TAA-TAG-AAC-GAC-TC	2353–2337	
P5	CGG-AAT-GCA-TAC-GGC-TC	2227–2243	P5–P6: 1298
P6	AGC-CAT-TAC-AGT-AAT-TA	3525–3509	
P7	GGA-TGG-ACT-AAC-ACC-AA	2769–2785	P7–P8: 1273
P8	TTA-AGT-ATA-ATT-CAA-CC	4042–4026	
P9	GTG-AAG-GGA-TTG-AAT-TG	3569–3585	P9–P10: 1224
P10	TCC-AAT-CCC-CAA-GTT-TC	4793–4777	
P11	AAA-CGA-CTA-TTC-CAA-AC	4675–4691	P11–P12: 1678
P12	CAT-AGT-ATA-ATC-GGC-AA	6353–6337	
P13	GTG-TGA-AAT-ATA-TTT-CT	6229–6245	P13–P14: 1792
P14	TTA-TCA-CCC-CTT-TAA-C	8021–8006	
P15	TTT-GGA-TTT-TGA-AAG-G	6979–6994	P15–P16: 1941
P16	GGA-TTT-ACT-ATT-ATC-AC	8920–8904	
P17	ATT-CAT-CTA-CAT-TGG-TG	8889–8905	P17–P18: 1584
P18	TCA-GTC-CAA-GAA-AGC-CT	10473–10457	
P19	TAT-CTT-CGC-TAT-TGG-AG	10403–10419	P19–P1: 427

[a] bp, Base pair.

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