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Comparison of an Automated Ribotyping System to Restriction Endonuclease Analysis and Pulsed-Field Gel Electrophoresis for Differentiating Vancomycin-Resistant *Enterococcus faecium* Isolates

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The RiboPrinter Microbial Characterization System was compared with pulsed-field gel electrophoresis (PFGE), restriction endonuclease analysis (REA), and epidemiological data for typing 45 vancomycin-resistant *Enterococcus faecium* (VRE) isolates. In 21 clinically related isolates, 90 to 100% were similar by PFGE and REA, but only 57% were similar by the RiboPrinter. In another eight clinically related isolates, three isolates similar by PFGE and REA were all unique by the RiboPrinter. In contrast, in 16 clinically unrelated isolates, the predominant RiboPrinter ribotype represented 50% of the strains, while the largest PFGE and REA clones represented less than 19% of the strains. These data suggest that the RiboPrinter is not reliable for VRE investigation.

Enterococci (especially those carrying vancomycin resistance genes) are important causes of clinical infections that can be spread nosocomially (10). In order to understand and control outbreaks, it is useful to determine genetic relatedness between human isolates of the same species (12).

Ribotyping, restriction endonuclease analysis (REA), and pulsed-field gel electrophoresis (PFGE) are among the most widely used of these methods for typing enterococci. PFGE has been shown to be useful for epidemiologic evaluations of nosocomial enterococcal infections (11), and most investigators consider PFGE to be the gold standard to which all other techniques are compared (16). REA and ribotyping have previously been compared to PFGE, and while REA and PFGE appear equally discriminatory (15), PFGE was found to be more discriminatory than traditional ribotyping for differentiating strains of *Enterococcus faecalis* (7). Nevertheless, ribotyping has been a useful typing technique for other organisms (2).

Due to its rapidity and ease of use, ribotyping has become more widely used as a fully automated technique. The RiboPrinter Microbial Characterization System (Qualicon, Wilmington, Del.) is one such automated instrument that performs ribotyping and uses computer analysis to compare ribotype profiles (3). The RiboPrinter system can automatically process up to eight bacterial isolates at one time, with results available about 8 h from sample input, and can accept new sample batches every 2 h. This system has been shown to perform adequately when compared to PFGE for typing a variety of organisms, including *Escherichia coli* and *Pseudomonas aeruginosa* (8, 14). In addition, *Listeria monocytogenes* (1), methicillin-resistant *Staphylococcus aureus* (6), *Campylobacter* spp. (5), as well as enterococci (9) have been typed for epidemiological purposes using this system.

We compared the RiboPrinter Microbial Characterization System with PFGE and REA as a means of typing clinically related and unrelated isolates of vancomycin-resistant *Enterococcus faecium* (VRE). As this automated technique has not yet been clinically validated, we assessed its utility for focusing infection control interventions based upon clinical correlation, as determined by epidemiologic data previously obtained.

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A total of 45 clinical and surveillance isolates of VRE that were obtained from 42 patients hospitalized at Northwestern Memorial Hospital, Chicago, Ill., during a 15-month period between July 1992 and October 1993 were recovered from storage at −70°C. Representative isolates were reanalyzed by REA using *Hin*dIII by the methods of Clabots et al. (4) and compared to the previously obtained REA typing results (15) for assurance that the strains recovered from frozen storage were accurately labeled.

All samples were ribotyped at the University of Iowa using the RiboPrinter Microbial Characterization System by the method of Bruce (3). To summarize, the automated process begins by lysing cells and cutting the released DNA into fragments with a restriction enzyme (*Eco*RI). These fragments are separated by size through gel electrophoresis and then transferred to a membrane, where they are hybridized with a DNA probe and mixed with a chemiluminescence agent. Each lane of sample is normalized to a standard marker set and band intensity. A digitizing camera captures the light emission as image data, from which the system extracts a RiboPrint pattern. This pattern is compared to others in the database for characterization and identification.

Similarity coefficients between the 45 isolates were calculated on the basis of band position, weight, and intensity. In-

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dividual isolates are judged to have the same RiboPrint pattern or ribotype if the similarity coefficient between the patterns is ≥0.93. The ribotypes were named according to the instrument number (first three digits), accession number (second three digits), first initial of the software program used (single letter), and run number (last single digit) of the first isolate identified with that RiboPrint pattern (3).

The ability of the RiboPrinter Microbial Characterization System to distinguish strains was compared with results previously determined on the same 45 VRE isolates by epidemiologic investigation, PFGE, and REA (15). For the REA typing, all the strains were analyzed twice at Northwestern Memorial Hospital, first with *Hae*III and then with *Hin*dIII. Similarities between REA types were scored by visual comparison of each 1-mm segment of the top 60 mm of the DNA band patterns run on the same gel by the method of Clabots et al. (4). Six or more differences over the top 60 mm constituted a similarity index of less than 90% and was designated as a new REA type. PFGE was previously performed on the same 45 enterococcal isolates at the University of Iowa by the method of Pfaller et al. (13). Briefly, macrorestriction digestion of genomic DNA was performed with *Sma*I, and the resultant PFGE patterns were considered identical if they shared every band, similar (subtype) if they differed from one another by one to three clearly visible bands, and distinct if they differed by over three bands. In both these schemes, letters designated the types, and numbers indicated a subtype designated a similar pattern within a type. All strains within a given type (designated by a letter) were considered related by the typing method for this investigation. Clinical correlation was obtained from results of the previously performed chart reviews on 42 patients (15). Simultaneous location on the same ward, same-day visits by consulting services, same-day common procedures, or presence in the same room within 3 days of another patient with VRE constituted clinical relatedness. Each of these investigations–epidemiologic investigation, PFGE, and REA–had been accomplished separately and independently without knowledge of the other results.

The previously obtained results of chart reviews (15) (chart reviews performed under the research guidelines of Northwestern University Medical School and Northwestern Memorial Hospital, Chicago, Ill.) enabled grouping of the patients into three distinct populations. There were two clusters of epidemiologically related isolates, comprising two separate outbreaks, and a third group of epidemiologically unrelated patients (15). Table 1 contains the data for these three patient groups and compares the results of the typing methods. Similarity coefficients between all 12 ribogroups found in these 45 isolates were assessed by the RiboPrinter and ranged from 0.41 to 0.97.

In the first outbreak of 21 clinically related strains, PFGE and REA results correlated well with the results of the chart reviews. All 21 clinically related samples were designated REA type B, and all but 2 were designated PFGE type B. However, two predominant RiboPrint patterns emerged in the first outbreak. The RiboPrinter Microbial Characterization System designated 8/21 isolates as one ribotype (105-187-S-4) and 12/21 isolates as another ribotype (105-271-S-6). The similarity coefficient between these two ribogroups was 0.88. To determine if these could be the same ribotype, the patterns from RiboPrint groups 105-187-S-4 and 105-271-S-6 were examined manually. After darkening the images, an extra band appeared in isolates EF13, EF15, and EF16, and a slight variation in band spacing between two bands and intensity appeared in EF12. However, manual examination did not improve correlation with REA or PFGE, as these differences did not result in regrouping of the isolates into a similar ribotype. Although REA and PFGE were useful for triggering an epidemiologic investigation in this cluster, the RiboPrinter results may have been too discriminatory to suggest investigation of this outbreak. Alternatively, one could argue that if the clinical suspicion for an outbreak were high, the finding of two predominant ribogroups from 21 isolates with a similarity coefficient of 0.88 should trigger an epidemiologic investigation.

In the second clinical outbreak of eight patients, all methods produced polyclonal results. The RiboPrinter Microbial Characterization System identified six unique types, PFGE identified six unique types, and REA identified three unique types with *Hin*dIII and five unique types with *Hae*III. However, EF21, EF22, and EF26 were all related clinically and by REA and PFGE but were distinct by the RiboPrinter. The similarity coefficients between each of the three corresponding ribotypes (105-271-S-6, 105-187-S-4, and 253-248-S-2) were 0.70 (105-187-S-4 and 253-248-S-2), 0.82 (105-271-S-6 and 253-248-S-2), and 0.88 (105-271-S-6 and 105-187-S-4). Here too, the RiboPrinter in its present form appeared too discriminatory, since it would have completely missed the potential association between these three isolates.

Paradoxically, the opposite was true for the clinically unrelated group of 16 isolates. In this case, the RiboPrinter Microbial Characterization System was the least-discrepant typing methods. The RiboPrinter identified only 7 distinct clonal types, with the predominant subtype representing 8 of 16, or half, of these strains, suggesting that patient-to-patient transmission had occurred, when there was no epidemiologic evidence to support this finding. In this same group, PFGE and REA methods identified between 8 and 14 unique types, with the largest genomic clone representing no more than 3 of 16, or less than 19%, of these VRE.

Similarity coefficients between distinct ribotypes as high as 0.97 demonstrates an example of the possible interpretative problems with the current ribotyping software. This very high similarity index of 0.97 was assigned for ribotypes 105-292-S-2 and 105-271-S-6. EF24, the corresponding isolate to 105-271-S-6, was not similar by PFGE or REA to any of the isolates corresponding to the 105-271-S-6 ribotype. Manual examination of the riboprinting patterns revealed that they differed by at least one band, thus leaving the ribogroup designation unchanged. Furthermore, the RiboPrinter Microbial Characterization System should categorize ribotypes with a similarity index of 0.97 and higher into the same ribogroup, but it did not in this case. The curious similarity coefficients could, in part, be explained by the fact that the RiboPrinter utilizes the average pattern among all strains it designates as belonging to a certain ribogroup. In this case, the RiboPrinter averaged the RiboPrint patterns of 14 isolates belonging to ribogroup 105-271-S-6 when calculating a similarity coefficient between that and ribogroup 105-292-S-2. Therefore, utilizing average RiboPrint patterns to calculate similarity coefficients may produce a de-

TABLE 1. Description of isolate sources and genomic typing result

Isolate	REA		PFGE	Ribotype	Date (mo/day/yr)	Unit[a]	Source
	*Hin*dIII	*Hae*III					
Outbreak 1							
EF6	B1	B1	B1	253-159-S-3	1/6/93	14E	Biliary
EF3	B0	B1	B1	105-187-S-4	12/2/92	15E	Wound
EF10	B0	B1	B1	105-187-S-4	2/5/93	MICU	IV catheter[b]
EF13	B3	B5	B5	105-187-S-4	3/2/93	14W	Toilet
EF15	B2	B3	B5	105-187-S-4	3/2/93	14W	Toilet
EF18	B2	B2	B5	105-187-S-4	2/23/93	14W	Rectum
EF25	B2	B2	B1	105-187-S-4	4/20/93	ER	Urine
EF32	B3	B3	I	105-187-S-4	5/22/93	7E	Urine
EF36	B4	B4	M	105-187-S-4	6/2/93	8E	Rectum
EF4	B0	B1	B1	105-271-S-6	12/18/92	MICU	Blood
EF5	B0	B1	B1	105-271-S-6	12/27/92	7W	Blood
EF8	B2	B2	B1	105-271-S-6	2/4/93	14W	Rectum
EF11	B2	B2	B3	105-271-S-6	2/23/93	SCICU	Rectum
EF12	B2	B3	B4	105-271-S-6	3/2/93	14W	Rectum
EF14	B0	B1	B1	105-271-S-6	3/4/93	SCICU	Hands
EF16	B2	B3	B1	105-271-S-6	3/2/93	SCICU	Rectum
EF17	B2	B3	B1	105-271-S-6	3/2/93	SCICU	Rectum
EF19	B2	B3	B1	105-271-S-6	2/26/93	10E	Chest tube
EF29	B2	B2	B1	105-271-S-6	5/10/93	MICU	Urine
EF30	B2	B2	B1	105-271-S-6	5/14/93	10E	Urine
EF31	B2	B2	B1	105-271-S-6	5/10/93	MICU	IV catheter
Outbreak 2							
EF20	C1	D0	D	105-187-S-4	3/21/93	8E	Urine
EF22	B2	B2	B7	105-187-S-4	4/7/93	SICU	Rectum
EF21	B2	B2	B6	105-271-S-6	3/15/93	8W	Urine
EF24	D0	F0	F	105-292-S-2	4/10/93	8E	Urine
EF23	B5	E0	E	253-247-S-7	4/7/93	SICU	Rectum
EF26	B2	B2	B1	253-248-S-2	4/22/93	SICU	Blood
EF27	B6	G1	G	253-248-S-3	4/21/93	14E	Rectum
EF28	B7	G1	H	253-248-S-3	4/21/93	14W	Rectum
Unrelated clinically							
EF2	B0	B0	B1	105-187-S-4	9/3/92	14W	Blood
EF7	B2	B2	B2	105-187-S-4	1/27/93	HH	Urine
EF35	B2	B2	L	105-187-S-4	5/18/93	14E	Rectum
EF38	F1	I0	B1	105-187-S-4	6/24/93	14E	Blood
EF39	D0	J0	O	105-187-S-4	7/22/93	11E	Rectum
EF41	D1	L0	Q	105-187-S-4	8/10/93	11E	Urine
EF44	H0	N0	T	105-187-S-4	9/27/93	12W	Wound
EF45	C1	D0	U	105-187-S-4	10/7/93	10W	Urine
EF42	F0	I0	R	105-271-S-6	7/27/93	10W	Urine
EF43	G0	M0	S	105-272-S-4	8/23/93	9W	Wound
EF1	A0	A0	A	253-245-S-1	7/2/92	14E	Blood
EF9	C0	C0	C	253-246-S-1	2/1/93	6W	Urine
EF37	E1	G0	N	253-248-S-3	6/8/93	14E	Rectum
EF33	E0	H0	J	253-249-S-1	5/18/93	14W	Urine
EF34	E0	H1	K	253-249-S-1	5/18/93	14E	Rectum
EF40	A1	K0	P	253-249-S-8	7/21/93	SCICU	Rectum

[a] Abbreviations: E and W, east and west wings, respectively; MICU, medical intensive care unit; SCICU, spinal cord intensive care unit; ER, emergency room; HH, home health.
[b] IV, intravenous.

ceptively high or low value relative to similarity coefficients between individual isolates.

Another source for inconsistency could lie in the relatively few bands available for typing enterococci compared to other methods. RiboPrint patterns for enterococci using *Eco*RI characteristically produce 8 to 9 bands, while PFGE using *Sma*I will produce at least 15 bands and REA using *Hae*III or *Hin*dIII will produce between 25 and 30 bands to be analyzed. Double restriction enzyme digestion using *Ase*I and *Bam*HI with this system has shown good discrimination compared to PFGE for strain characterization of VRE (A. B. Turlak, E. Cole, B. Brinton, L. Eutropius, M. Samore, and K. C. Carroll, Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001, abstr. C-83, p. 167, 2001), and perhaps this practice would have improved correlation in our hands as well. The RiboPrinter Microbial Characterization System also considers the weight and intensity of the bands when isolate comparisons are performed, something not included in the analysis for either REA or PFGE. While it is unclear which of these unique interpretative approaches applied to the RiboPrinter may have lead to the differences in

relatedness interpretations compared to PFGE and REA, further analysis of them may be useful in future RiboPrinter software enhancements.

Although most available DNA-based typing methods may be used in studying nosocomial infections when applied in the context of a careful epidemiologic investigation, even the most powerful and sophisticated typing method, if used indiscriminately in the absence of sound epidemiologic data, can provide conflicting and confusing information. In summary, using the RiboPrinter Microbial Characterization System for determining the relatedness of these 45 VRE isolates produced inconsistent results that were both too discriminatory and not discriminatory enough to be useful in this epidemiologic investigation. Program modifications, perhaps double restriction enzyme digestion, may be needed to enhance the utility of this highly automated system when typing VRE for epidemiologic purposes.

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