Typing of *Enterococcus* spp. strains isolated from patients with infective endocarditis by an automated repetitive-sequence-based PCR system

Giuseppe Russello[1], Claudio Farina[1], Annibale Raglio[2], Veronica Ravasio[3], on behalf of the Italian Infective Endocarditis Study Group (SEI)[4]

1 UOC Microbiologia e Virologia, AO "Ospedale San Carlo Borromeo", Milano, Italy
2 USC Microbiologia e Virologia, AO "Ospedali Riuniti di Bergamo", Bergamo, Italy
3 USC Malattie Infettive, AO "Ospedali Riuniti di Bergamo", Bergamo, Italy
4 SEI group: Suter F, Rizzi M, AO Ospedali Riuniti di Bergamo; Biglino A, Ospedale Civile di Asti; Carosi G, Università degli Studi di Brescia; Persiani M, Ospedale di Cuneo; Utili R, AO Monaldi, Napoli; Tripodi MF, II Univ., Policlinico Universitario di Napoli; Minoli L, IRCCS – Policlinico San Matteo, Pavia; Petrosillo N, INMI "L. Spallanzani", Roma; Venditti M, Policlinico Umberto I, Roma; Vaglia A, Ospedale S. Maria di Ca' Foncello, Treviso; Crapis M, AOU 'S. Maria della Misericordia', Udine; Grossi P, Università degli Studi dell'Insubria, Varese; Concia E, Università degli Studi di Verona.

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SUMMARY

The use of rep-PCR was studied to characterize 32 isolates of *Enterococcus* spp. from Italian patients with infective endocarditis and determine specific banding patterns associated with antimicrobial resistance phenotypes.

The results obtained with rep-PCR were analyzed with respect to the antimicrobial profile by a MIC evaluation (E-test) of all strains tested.

The automated rep-PCR technique resulted an easy method for the characterization of *Enterococcus* spp. It confirmed the eterogenicity of enterococcal strains involved in infective endocarditis.

Furthermore results of antimicrobial susceptibility revealed that rep-PCR can not be used as diagnostic tool to characterize and identify difference between resistant and susceptible *Enterococcus* spp. strains.

The semi-automated repetitive-sequence-based PCR (rep-PCR) is a method that has been standardized and partially automated to type the microbial strains scattering throughout the microbial genome.

The main aim of our study was to investigate the utility of using rep-PCR to correlate the typing profile of a medically-important group of bacteria like *Enterococcus* species, considering this homogeneous group of strains from patients affected by infective endocarditis. The secondary aim was to verify a possible correlation between the genotypic profile and the chemosensitivity pattern.

We analyzed 32 *Enterococcus* strains (*E. faecalis*, 27; *E. faecium*, 5) isolated from Italian patients affected by infective endocarditis, included in the Italian Infective Endocarditis Survey.

Enterococcus faecalis ATCC 29212 was used as internal control.

All strains were analyzed by the rep-PCR technique. It separates amplicons and generates fingerprinting patterns at microbial subspecies level (4-6) after a genomic DNA extraction (UltraClean™ *kit,* Mo Bio, Laboratories, Solana Beach, CA, USA), amplification (DiversiLab™ *Enterococcus* Kit) and pattern generation by microfluidic chips (LabChip device; Caliper Technologies, Inc.) read through a bioanalyzer (Agilent 2100 Bioanalyzer™, Agilent Technologies, Palo Alto, CA, USA).

The results are then performed with DiversiLab software (version 2.1.6.6), which uses Pearson correlation coefficient to determine distance matrices and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) to create dendrograms. Patterns are automatically downloaded onto a laboratory-specific DiversiLab website for analysis and interpretation. Both gel-like images and dendrograms are created for comparative analysis.

Reports were automatically generated as dendrogram, electropherograms, virtual gel images and scatter plots. According to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM) guidelines (7), two strains that differ by one or more bands, irrespectively from their intensity, are considered unrelated. The

Corresponding author: **Claudio Farina**
UOC Microbiologia e Virologia, AO "Ospedale San Carlo Borromeo"
Via Pio II 3 - 20153 Milano - Tel +39 02 40222456; Fax +39 02 40222829
E-mail: **farina.claudio@sancarlo.mi.it**

intensity of the bands was not evaluated, since we did not perform replicate experiments.

The Minimal Inhibitory Concentration (MIC) of all strains was determined by an agar diffusion gradient method (E-test, bioMérieux, Marcy-l'Etoile, France) for ampicillin (0.016-256 mg/L), high dosage gentamycin and streptomycin (0.064-1024 mg/L), imipenem (0.002-32 mg/L), ceftriaxone (0.016-256 mg/L), vancomycin (0.016-2 mg/L), linezolid (0.016-256 mg/L), daptomycine (0.016-256 mg/L), tigecycline (0.016-256 mg/L), rifampicin (0.002-32 mg/L), fosfomycin (0.064-1024 mg/L), and moxifloxacin (0.002-3 mg/L).

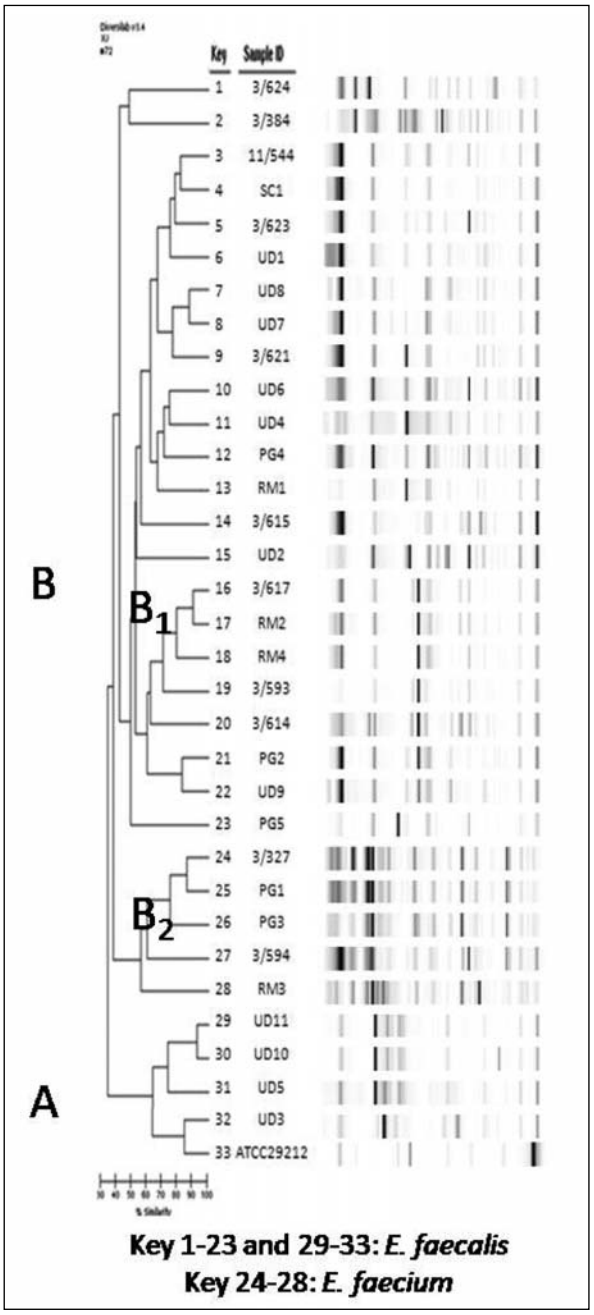

Figure I. *Report of* Enterococcus *spp. DiversiLab System. From left to right lines: 1. Dendrogram; 2. Sample ID; 3. Virtual gel image fingerprints.*

Figure I summarizes the rep-PCR report.

The ATCC 291121 strain, used as internal control resulted not related to the other strains tested.

The dendrogram analysis shows the different groups of isolates. Two strains (strains n. 29 and 30) appear strictly related (average similarity ratio: 92.4%) even if the graphics' overlay confirms a little difference at the fingerprinting pattern (Figure II). Interestingly, both the strains have been recovered from the same patient from blood cultures collected at the baseline (t_0) and two months later (t_1). In particular, both strains present the same chemosensitivity profile except for fosfomycin (MIC value of t_0 strain: 24 mg/L; MIC value of t_1 strain: 94 mg/L). Isolates at lanes 24-28 are *E. faecium*, whereas all the other strains are *E. faecalis*. All the strains included in the study appear to be not related, except for the two isolates described above. No correlation between chemosensitivity pattern and rep-PCR typing pattern has been found (Table 1).

The automated repetitive-sequence-based PCR system (rep-PCR) is based on non-coding repetitive sequence elements within the genome of organisms to create primers and amplify regions between these sequences. Fragments of various lengths are generated and separated by microfluidic electrophoresis. In the Agilent system, the fragments pass over a laser as they separate, creating a graph of fluorescence intensity over time. This graph translates to the fingerprint pattern.

The rep-PCR appears specific for *Enteroccus* spp. and it could be used also to differentiate enterococcal strains belonging to different species. As expected, an homogeneous subcluster includes only *E. faecium* isolates.

Our study confirm that the technique is highly reliable, as demonstrated by the fact that two similar profiles were originated by two strains collected from the same patient.

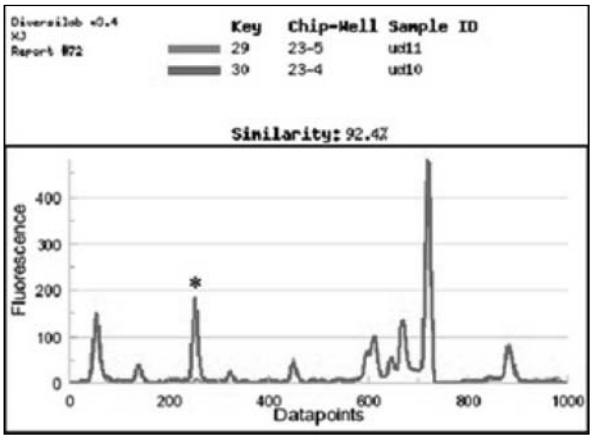

Figure II. *Electropherogram overlay of rep-PCR amplicons from strains n°29 and 30. The arrow indicates a fluorescent peak difference between the samples.*

Table 1. In vitro *antimicrobial susceptibility for* Enterococcus *spp. strains representative of each pattern obtained by rep-PCR.*

Sample ID	Pattern	Strains	AMP	HGM	HST	IMP	CRO	VA	LZ	DAP	TIG	RA	MOX	F
3/624	1	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
3/384	2	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
11/544	3	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
SC01	4	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
3/623	5	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	R
UD1	6	*E. faecalis*	S	S	R	S	R	S	S	S	S	S	S	S
UD8	7	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
UD7	8	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
3/621	9	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
UD6	10	*E. faecalis*	S	R	S	S	R	S	S	S	S	R	R	S
UD4	11	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	R	S
PG04	12	*E. faecalis*	S	R	S	S	R	S	S	S	S	S	R	R
RM1	13	*E. faecalis*	S	S	S	S	R	S	S	S	S	R	S	S
3/615	14	*E. faecalis*	S	S	R	S	R	S	S	S	S	S	S	S
UD2	15	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
3/617	16	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
RM2	17	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
RM4	18	*E. faecalis*	S	S	R	S	R	S	S	S	S	S	S	S
3/593	19	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
3/614	20	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
PG02	21	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
UD9	22	*E. faecalis*	S	S	R	S	R	S	S	S	S	S	S	S
PG05	23	*E. faecalis*	S	R	R	S	R	S	S	S	S	S	R	S
3/327	24	*E. faecium*	R	S	S	R	R	S	S	S	S	R	R	S
PG01	25	*E. faecium*	R	R	R	R	R	R	S	S	S	R	R	S
PG03	26	*E. faecium*	R	S	R	R	R	R	S	S	S	S	R	S
3/594	27	*E. faecium*	R	R	R	R	R	R	S	S	S	R	R	S
RM3	28	*E. faecium*	R	S	S	R	R	S	S	S	S	S	S	S
UD11	28	*E. faecalis*	S	S	S	S	R	S	S	S	S	R	S	I
UD10	30	*E. faecalis*	S	S	S	S	R	S	S	S	S	R	S	S
UD5	31	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
UD3	32	*E. faecalis*	S	S	S	S	R	S	S	S	S	S	S	S
	33	ATCC 29212	S	S	S	S	R	S	S	S	S	S	S	S

While one can expect organisms with similar antimicrobial profiles to cluster together, it has not been shown to be useful for identification of specific antimicrobial resistance mechanisms.

In conclusion, rep-PCR can be proposed as an useful method to type also *Enterococcus* spp. It could be utilized in case of nosocomial outbreaks, as described also for methicillin resistant *Staphylococcus aureus* (MRSA) or Gram negative (5, 1). As expected, our results confirm the wide eterogenicity of enterococcal strains involved in infective endocarditis.

No correlation can be described between chemosensitivity and ribotyping pattern, confirming the results of Chuang et al. (2010), even if it can be useful for a quick strains' classification versus the reference strains (3).

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