

Random Amplified Polymorphic DNA Typing of *Enterococcus faecalis* Isolated from Lebanese Individuals

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Objective: In this study we assessed the utility of random amplified polymorphic DNA in the subtyping of 38 isolates of *Enterococcus faecalis*.

Method: Twenty of 38 subtypes were recovered from urinary tract infections and 18 from rectal swabs of hospitalized patients with no enterococcal infections. All isolates were initially identified by conventional bacteriological methods and the API 20 Streptococcus system. Biotyping was done on the basis of hippurate hydrolysis (HH) test using API 20S. Susceptibility of the isolates to vancomycin, teicoplanin, ampicillin and gentamicin was performed by the disk diffusion and the agar dilution methods. Antimicrobials were selected based on treatment regimen adopted by clinicians. DNA was extracted from all isolates using the PureGene kit and Random Amplified Polymorphic DNA Typing (RAPD) was done using a 10 mer and 22 mer primers reflecting the GC content of the genome.

Results: Our data have shown that 38 tested isolates were *E. faecalis*. Thirteen of 38 were of fecal origin and 6/38 were of urine origin. All 19 isolates were of biotype I (HH positive). The remaining 5 of fecal origin and 14 of urine origin were of biotype II (HH negative). All isolates were susceptible to vancomycin, teicoplanin and ampicillin except 3 urine and 1 fecal isolates that showed resistance to ampicillin. All urine isolates were resistant to gentamicin while 3 of 18 fecal isolates were susceptible to this antimicrobial agent. RAPD data have shown that 2/19 biotype I isolates, showed one RAPD pattern and were susceptible to teichoplanin and vancomycin; and 2/19 showed a different pattern and were susceptible to all tested antimicrobials except gentamicin. All 4 isolates were of urine origin. The remaining 15 biotype I isolates had different patterns. In addition, of 19 biotype II isolates, 2 showed one pattern and the remaining 17 different patterns. Two isolates were of urine origin and resistant to gentamicin. The RAPD patterns were reproducible.

Conclusion: These observations suggest that RAPD is efficient for subtyping *E. faecalis* and may be useful as a tool in epidemiologic investigations.

Key words: RAPD, *enterococcus faecalis*, subtyping.

Enterococci are part of the normal gastrointestinal flora of all warm-blooded animals including humans (1). They can enter the human food chain from animal reservoirs (2). Although *Enterococci* are considered to be of low viru-

lence, they are recognized as important causes of nosocomial infections (3). *Enterococcus faecalis* and *Enterococcus faecium*, the most common human isolates among the *Enterococci*, are encountered as etiological agents of urinary tract infections, wounds, endocarditis, bacteremia, and intraabdominal abscesses (4,5). Several methods are introduced to type the phenotypic characteristics and to investigate the source and the mechanisms of spread of *Enterococci*. The aim of the study was to determine the usefulness of random amplified polymorphic DNA typing (RAPD) in the subtyping of *E. faecalis* with known biotypes based on hippurate hydrolysis assay. We analysed 38 isolates recovered from urine and rectal swab specimens. RAPD with primers AP4 and ERIC1R was able to identify 32 different DNA patterns on isolates belonging to different biotypes.

Material and Method

In this report and in an attempt to assess the utility of RAPD in the subtyping of *Enterococcus faecalis*, a total of 38 isolates were collected and studied. Of the isolates, 20 were recovered from outpatients with urinary tract infections as determined by colony count and clinical diagnosis, and 18 from rectal swabs of hospitalized patients with non-Enterococcal infections admitted to the American University of Beirut Medical Center (AUBMC). The urine isolates were recovered on Trypticase soy blood agar (BBL, Cockeysville, MD), while the rectal swab isolates were recovered on Phenylethyl alcohol blood agar (BBL, Cockeysville, MD). All isolates were initially identified as enterococcus species by Gram staining, and on the basis of a positive reaction for bile esculine hydrolysis, growth in 6.5% NaCl, and growth and acid production in SF (*Streptococcus faecalis*) medium (Biolife, Milano, Italy). Identification of the *Enterococci* was confirmed using the API 20 Streptococcus system (Biomerieux, Marcy Etoile, France) and, biotyping of the isolates was done on the basis of hippurate hydrolysis (HH) test. Antimicrobial susceptibility of the isolates to vancomycin, teicoplanin, gentamicin, and ampicillin was tested by the disk diffusion and agar dilution methods as recommended by the National Committee for Clinical Laboratory Standards (11,12). DNA was extracted from all isolates using the Pure Gene kit (Gentra Systems, Inc. NC), and (RAPD) was done using the primers AP4 (5' TCA CGC TGC A 3')

and ERIC1R (5 ATG TAA GCT CCT GGG GAT TCA C 3) (1), according to Matar et al (7).

Results

The isolates were identified as *E. faecalis* based on the API profile determined after 4 hours, and 24 hours of incubation. Of the 38 isolates, 19 were of biotype I (HH positive), which included 13 isolates of fecal and 6 of urine origin. The other 19 isolates belonged to biotype II (HH negative), which included 5 isolates of fecal origin and 14 of urine origin. The susceptibility and minimal inhibitory concentration (MIC) values for all isolates are shown in tables I,II. All isolates proved to be susceptible to vancomycin and teicoplanin. One fecal isolate and 3 urine isolates demonstrated resistance to ampicillin, and had a MIC value of 64m/ml. All urine isolates were resistant to gentamicin, 4 of which had a MIC value of ³1000mcg/ml while 15 of 18 fecal isolates were resistant to this antimicrobial agent, 1 of which had a MIC value of ³1000mcg/ml. RAPD data have shown that 2/19 biotype I isolates, showed one RAPD pattern and were sensitive to teicoplanin and vancomycin; and 2/19 a different pattern and were susceptible to all tested antimicrobials except gentamicin. All the 4 isolates were of urine origin. The remaining 15 biotype I isolates had different RAPD patterns. In addition, 2/19 biotype II isolates showed one RAPD pattern and the remaining 17 isolates showed different RAPD patterns. The 2 isolates were of urine origin and resistant to gentamicin. The RAPD patterns of the tested isolates were all reproducible. Figure 1. shows few representative RAPD patterns. The heterogeneity observed among these isolates relates to the diversity of their sources. It also suggests that RAPD is recommended for subtyping *E. faecalis* and may be useful as a tool in epidemiologic investigations if laboratory information is coupled with adequate epi-data and hence may be useful in tracing the source of *E. faecalis* implicated in various infections.

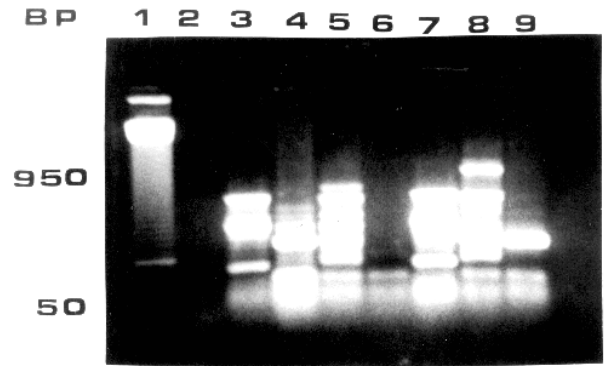


Figure 1. Representative RAPD patterns of *E. faecalis* Lane 1: 50 bp-ladder, lane 2: Negative Control, lane 3-9: Representative RAPD, RAPD patterns.

Discussion

This observation indicates that RAPD is discriminatory and able to identify multiple strains implicated in urinary tract infections. This is consistent with a previous study investigating the use of RAPD in subtyping vancomycin resistant *E. faecium*. The heterogeneity detected in these isolates may be due to the diversity of their sources. It also suggests that RAPD is useful for subtyping *E. faecalis* and maybe adequate as an epidemiologic marker in tracing the source of various types of infections caused by this organism.

Several typing methods that are based on phenotypic characteristics have been adopted to investigate the source and the mechanisms of spread of *Enterococci*. These include biochemical reactions, antibiotic resistance patterns, serological typing, and bacteriocin typing. Such methodologies, however, did not prove to be sufficiently discriminative (6). Recently, genotypic characterization of strains have been increasingly used to determine the relevance of strains especially in vancomycin resistant *E.*

Table I. In vitro activity of four antimicrobial agents against 20 *E. faecalis* isolated from urine

Biotype (n)	Number of isolates inhibited at indicated concentration or concentration range (μ)										
	Ampicillin			Gentamicin			Teicoplanin		Vancomycin		
	<1	2-4	64	<4	8-32	>1000	<0.125	0.25-0.5	<1	1-2	4
Biotype I (6)	1	3	2	-	4	2	-	6	2	4	-
Biotype II(14)	5	8	1	-	12	2	-	14	1	12	1

Table II. In vitro activity of four antimicrobial agents against 18 *E. faecalis* isolated from rectal swabs.

Biotype (n)	Number of isolates inhibited at indicated concentration or concentration range (μ)										
	Ampicillin			Gentamicin			Teicoplanin		Vancomycin		
	<1	2-4	64	<4	8-32	>1000	<0.125	0.25-0.5	<1	1-2	4
Biotype I (13)	13	-	-	3	10	-	11	2	-	11	2
Biotype II(5)	1	3	1	-	4	1	4	1	-	5	-

faecium. Of the methods used are the total plasmid content, ribotyping, and DNA fingerprinting by the pulsed field gel electrophoresis (PFGE). The latter is considered as the most discriminative typing technique, however it requires specific equipment and is time consuming (2,5). Recently, RAPD was successfully used to type different microorganisms such as *Clostridium difficile*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, and *Enterococcus faecium* (2,5,7-10). The advantage of this method is that no prior sequence information about the target is needed and restriction analysis is not required. It was shown to be well suited for epidemiological typing of strains in addition to being quicker and easier to perform than PFGE.

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