

Identification and Biochemical Reactions of Enterococci by a Simplified Identification System

Young Uh, In Ho Jang, Gyu Yel Hwang, Kap Jun Yoon and Hyung-Hoan Lee*

Department of Clinical Pathology, Yonsei University Wonju College of Medicine, Wonju, Korea,
and Department of Biology, Kon-Kuk University, Seoul, Korea*

Background : The accurate and rapid identification of enterococci can provide clinician's decision making of antimicrobial therapy because enterococci are usually multiresistant to commonly used antimicrobial agents and antimicrobial resistance patterns are different according to enterococcal species. Accuracy of identification system depends mainly on data base such as positive rate of biochemical reactions, relative frequency of occurrence of biotype, and isolation frequency of microorganisms. The purpose of this study was to analyze the isolation rate and biotype frequency of enterococci isolated from clinical specimens.

Methods : We used a simplified identification system for the identification of the enterococci from clinical specimens during the period of June 1998 to November 1998. Biochemical phenotypes of 500 isolates of enterococci were also analyzed by a simplified identification system consisting of eight conventional biochemical tests.

Results : Enterococci were isolated from urine (36.4%), wound (35.0%) and blood (7.2%) in order of decreasing frequency. Among the isolates, 67.8% were *E. faecalis*, 23.0% *E. faecium*, 2.2% *E. hirae/durans*, 2.0% *E. casseliflavus*, and 1.0% *E. hirae*. The simplified identification system of enterococci identified 93.6% of all isolates to species level. The system identified 98.5% of *E. faecalis* but only 89.6% of *E. faecium*.

Conclusions : Our simplified identification system based on eight biochemical tests offer a simple, reliable and economic method for the identification of clinical isolates of enterococci, but further studies are needed for the improvement of accuracy and identification rate.

(Korean J Clin Microbiol 1999;2:58~63)

Key words : Enterococci, *Enterococcus*, Identification

: 1999 1 12 : CM99-6
: 1999 2 22
:
(220-701) 162

: 0371-741-1593 Fax : 0371-731-0506
E-mail : u931018@yonsei.wonju.ac.kr, U931018@hitel.net

Enterococcus () catalase

가

가 [1].

[2] 1970 가 가

1990

가 [1-3], 가 [4-8].

가

[1,2,9].

가 가 가 [10-13].

가 [14,15].

8

[16] , 6

Table 1. Isolation frequency of the enterococci

Species	No. of isolates accroding to specimens												No.(%) of total
	BI	BL	CA	CF	CX	DR	PE	PL	PS	UR	WD	OT	
<i>E. faecalis</i>	7	20	19	1	7	10	3	7	13	119	128	5	339(67.8)
<i>E. faecium</i>	7	13	2	0	0	4	2	3	0	55	29	0	115(23.0)
<i>E. hirae/durans</i>	0	0	0	0	1	0	0	0	0	3	7	0	11(2.2)
<i>E. casseliflavus</i>	3	2	1	0	0	0	0	0	0	0	4	0	10(2.0)
<i>E. hirae</i>	0	1	0	0	2	0	0	0	0	0	2	0	5(1.0)
<i>E. avium</i>	0	0	0	0	0	0	0	0	1	0	1	0	2(0.4)
<i>E. gallinarum</i>	1	0	0	0	0	0	0	0	0	0	1	0	2(0.4)
<i>E. raffinosus</i>	0	0	0	0	0	1	0	0	0	1	0	0	2(0.4)
<i>Enterococcus</i> sp.	0	0	0	0	5	0	1	0	1	4	3	0	14(2.8)
Total No.	18	36	22	1	15	15	6	10	15	182	175	5	500(100.0)
%	3.6	7.2	4.4	0.2	3.0	3.0	1.2	2.0	3.0	36.4	35.0	1.0	

Abbreviations: BI, bile; BL, blood; CA, catheter; CF, cerebrospinal fluid; CX, cervix; DR, drain; PE, peritoneal fluid; PL, pleural fluid; PS, pus; UR, urine; WD, wound; OT, other specimens.

1998
6 11 6 [16]
500
NaCl-esculin
가 , 0.04% tellurite , arginine
dihydrolase (ADH),
arabinose raffinose , methyl- α
-D-glucopyranoside (MAG
), pigment
8가 . NaCl-esculin Qadri
[17] , MAG Devriese [18] ,
[19]
bionumber가
catalase NaCl-esculin
API 20 STREP
(bioMerieux Vitek, France)
Enterococcus species ()
vancomycin API 20 STREP
(bioMerieux Vitek, France) 가
NCCLS [20]
vancomycin (30 μ g), ampicillin (10 μ g), gentamicin
(120 μ g), tetracycline (30 μ g) ciprofloxacin (5 μ g)
. Tetracycline ciprofloxacin
β -lactamase 가
gentamicin 가
[21], β -lactamase cefinase
(BBL Microbiology Systems, Cockeysville, MD., USA)
(36.4%), (35.0%),
(7.2%)
(catheter) (bile) 4.4% 3.6%(18/500)
,

Table 2. Biochemical reactions of enterococci

Species	Biochemical reactions								ID code	No.(%) of phenotype
	HHB	N-E	TEL	ADH	ARA	RAF	MAG	PIG		
<i>E. faecalis</i>	-	+	+	+	-	-	-	-	610	267(78.7)
	+	+	+	+	-	-	-	-	710	65(19.2)
	+	+	-	+	+	-	-	-	330	1(0.3)
	+	+	+	+	-	+	-	-	750	1(0.3)
									K	5(1.5)
<i>E. faecium</i>	-	+	-	+	+	-	-	-	230	51(44.4)
	-	+	-	+	+	+	-	-	270	52(45.2)
									K	12(10.4)
<i>E. hirae/durans</i>	-	+	-	+	-	-	-	-	210	11(100)
<i>E. casseliflavus</i>	-	+	-	+	+	+	+	+	273	7(70.0)
	-	+	-	-	+	+	+	+	263	1(10.0)
	-	+	+	-	+	+	+	+	663	1(10.0)
	-	+	-	-	-	+	+	+	243	1(10.0)
<i>E. hirae</i>	-	+	-	+	-	+	-	-	250	5(100)
<i>E. avium</i>	-	+	-	-	+	-	+	-	221	2(100)
<i>E. raffinosus</i>	-	+	-	-	+	+	+	-	261	2(100)
<i>E. gallinarum</i>	-	+	-	+	+	+	+	-	271	1(50.0)
									K	1(50.0)
<i>Enterococcus</i> sp.									K	14(100)

Abbreviations: ID, identification; HHB, hemolysis on 5% human blood agar plate; N-E, NaCl-esculin hydrolysis test; TEL, 0.04% tellurite tolerance test; ADH, arginine dihydrolase; ARA, arabinose; RAF, raffinose; MAC, methyl- α -D-glucopyranoside; PIG, pigment; +, positive reaction or β hemolysis; -, negative reaction or non- β hemolysis; K, identification by commercial kit system.

2.0%, 1.2% 0.2% (Table 1).
Enterococcus faecalis, *Enterococcus faecium*,
Enterococcus hirae/ *durans*, *Enterococcus casseliflavus* 1990 가
Enterococcus hirae 67.8%, 23.0%, 2.2%, [4].
2.0% 1.0% *Enterococcus avium*, *Enterococcus* ,
raffinosis *Enterococcus gallinarum* 2
14 (2.8%)
E. faecalis, *E. faecium*, *E.* .
casseliflavus *E. hirae* 55.6%(20/36),
36.1%(13/36), 5.6%(2/36) 2.8%(1/36) (Table 1). *E.* 가
faecalis , 가
37.8%, 35.1% 5.9% , *E. faecium* .
, 47.8%, 25.2%
11.3% (Table 1). NaCl-esculin 가 4
8가
93.6%(468/500) *E. faecalis*
98.5%(334/339) *E. faecium* 89.6% 가 ,
(103/115) . *Lactococcus*, *Vagococcus*, *Leuconostoc*, *Pediococcus*,
가 468 *Tetragenococcus*, *Aerococcus*, *Helcococcus*, *Streptococcus*
334 *E. faecalis* 1 *bovis* .
tellurite 1 14
arabinose , 67 (21%) (Table 1) 가 .
. 103 *E. faecium* raffinose
50.1% . *E. casseliflavus* tellurite 가 . 0.04%
, ADH arabinose 10%, tellurite *E. faecalis*
70% 90% (Table 2). *E. faecalis* 7-10%
E. faecalis *E. faecium* ampicillin [14,22], *E. casseliflavus*, *E. faecium*, *E.*
1.2% 77.9%, vancomycin 0.3% 11.5%, *gallinarum* *E. mundtii* [22,23]

Table 3. Antimicrobial resistance rate of enterococci

Antibiotics	No.(%) of resistant strains									
	EFA (n=339)	EFM (n=113)	EHD (n=11)	ECA (n=10)	EH1 (n=5)	ERA (n=2)	EGA (n=2)	EAV (n=1)	ENT (n=7)	Total (n=490)
AMP	4(1.2)	88(77.9)	5(45.5)	0(0.0)	2(40.0)	1(50.0)	0(0.0)	0(0.0)	2(28.6)	102(20.8)
VAN	1(0.3)	13(11.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(14.3)	15(3.1)
CIP	41(31.1)	43(79.6)	2(50.0)	NT	NT	1(100)	NT	NT	3(75.0)	90(46.2)
TET	109(82.6)	29(53.7)	2(50.0)	NT	NT	1(100)	NT	NT	4(100)	145(74.4)
HLG	190(56.0)	92(81.4)	7(63.6)	2(20.0)	2(40.0)	2(100)	0(0.0)	1(100)	3(42.9)	299(61.0)
BLA	1(5.0)	0(0.0)*	NT	0(0.0)*	NT	NT	NT	NT	NT	1(2.9)

*Twelve *E. faecium* and two *E. casseliflavus* were tested for β -lactamase test.

Abbreviations: EFA, *E. faecalis*; EFM, *E. faecium*; EHD, *E. hirae/durans*; ECA, *E. casseliflavus*; EH1, *E. hirae*; ERA, *E. raffinosus*; EGA, *E. gallinarum*; EAV, *E. avium*; ENT, *Enterococcus* species; AMP, ampicillin; VAN, vancomycin; CIP, ciprofloxacin; TET, tetracycline; HLG, high level gentamicin; BLA, β -lactamase; NT, not tested.

ciprofloxacin 31.1% 79.6%, gentamicin 56.0% 가 .
81.4% tetracycline 82.6% 53.7% tellurite
E. faecalis 1 β -lactamase .
(Table 3). *Enterococcus malodoratus*, *Enterococcus*
pseudoavium, *Enterococcus mundtii*, *Enterococcus dispar*
Enterococcus sulfureus . *E.*

faecalis *E. faecium* 67.8% 23.0%가
[23] [13] *E. faecalis*
5-9% *E. faecium* 4-11% , *E.*
casseliflavus, *E. hirae/durans*, *E. hirae* *E. raffinosus*
2.0%, 2.2%, 1.0% 0.4%
[9,13,23] *E. raffinosus* *E.*
casseliflavus, *E. hirae/durans* *E. hirae*
.
Enterococcus durans, *Enterococcus*
cecorum, *Enterococcus columbae* *Enterococcus*
saccharolyticus *E. casseliflavus* *E.*
flavescens

Vagococcus fluvialis *Lactococcus garveiae*
database가
mannitol, sucrose, sorbitol ribose 가

가 .
1.0 [23] 1.8
[13] 1.1 , 1990
[9] 0.7 , 7.2%
[13] 11.3% [23]
4% [9] 0.1% .

가
aminoglycoside, vancomycin, ampicillin
가 . 1984-1992
ampicillin
1984-1987 97% [24], 1988-
1992 80%가 [25], *E. faecalis*
E. faecium ampicillin 1992 0% 79%
[26] 1995-1997 0% 91%
가
가 [4]. β -lactam
PBP (Penicillin binding protein)
가 β -lactamase
[4]. β -lactamase
1983 Murray

Mederski-Samoraj[27]가 β -lactamase *E.*
faecalis

β
-lactamase *E. faecalis* 가
[4]. *E. faecium*
ampicillin 78% [4] 1997
93% *E. faecalis* ampicillin
1.2% 1 *E.*
faecalis β -lactamase . vancomycin
(VRE) 1992
[4]. VRE
3.1% [28] 2.3%
[29] 4.6% .

VRE *E. faecium* 86.7%(13/15) [28]
9 VRE *E. casseliflavus*가 5 가
vancomycin *E. faecium* , [29]
VRE *E. faecium*, *E. gallinarum*, *E.*
casseliflavus 33.3%, 19.4% 0.8%
VRE 가

;
 , 가

1998 6 11
8가

500

:(36.4%),
(35.0%) (7.2%)

E. faecalis, *E. faecium*, *E. hirae/durans*, *E. casseliflavus* *E.*
*hirae*가 67.8%, 23.0%, 22%, 2.0%, 1.0% .
93.6%
E. faecalis 98.5%
E. faecium 89.6%

;
가

가

1. Facklam RR and Sahm DF. *Enterococcus*. In: Murray PR, et al. eds. *Manual of clinical microbiology*. 6th ed. Washington DC: American Society for Microbiology, 1995:308-14.

2. Greenwood D. Antibiotic resistance in enterococci. *J Antimicrob Chemother* 1989;24:631-5.

3. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med* 1991;91:79S-82S.

4. . , 1998:141-70.

5. .

- (1996). : , 1997:1-104.
6. . 1996;36: 331-40.
7. , , , , . 1984-1993 . 1996;28:151-65.
8. , , , . 1995;27:379-86.
9. , , . *Enterococcus* . 1992; 27:103-14.
10. Ruoff KL, de la Meza L, Murtagh MJ, Spargo JD, Ferraro MJ. *Species identities of enterococci isolated from clinical specimens. J Clin Microbiol* 1990;28: 435-7.
11. Sader HS, Biedenbach D, Jones RN. *Evaluation of Vitek and API 20S for species identification of enterococci. Diagn Microbiol Infect Dis* 1995;22:315-9.
12. , , , , , . *Vancomycin Enterococcus faecium plasmid DNA random amplified polymorphic DNA* . 1998;18:379-85.
13. . *Aminoglycoside enterococci* . 1993; 13:419-27.
14. Facklam RR. *Recognition of group D streptococcal species of human origin by biochemical and physiologic tests. Appl Microbiol* 1972;23:1131-9.
15. Facklam RR and Elliott JA. *Identification, classification, and clinical relevance of catalase-negative gram-positive cocci, excluding the streptococci and enterococci. Clin Microbiol Rev* 1995;8:479-95.
16. , , . 1999;19:().
17. Qadri SM, Flournoy DJ, Qadri SG. *Sodium chloride-esculin hydrolysis test for rapid identification of enterococci. J Clin Microbiol* 1987;25:1107-8.
18. Devriese LA, Pot B, Kersters K, Lauwers S, Haesebrouck F. *Acidification of methyl- α -D- glycopyranoside: a useful test to differentiate Enterococcus casseliflavus and Enterococcus gallinarum from Enterococcus faecium species group and from Enterococcus faecalis. J Clin Microbiol* 1996;34:2607-8.
19. , . 2 . : , 1993:111-22.
20. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing; Eighth informational supplement. M100-S8. Villanova, Pa.: National Committee for Clinical Laboratory Standards, 1998.*
21. Leitch C and Boonlayangoor S. *Tests to detect high-level aminoglycoside resistance in enterococci. In Isenberg HD, ed. Clinical microbiology procedures handbook. Washington DC: American Society for Microbiology, 1992;5.4.1-7.*
22. Facklam RR and Collins MD. *Identification of Enterococcus species isolated from human infections by a conventional test scheme. J Clin Microbiol* 1989;27: 731-4.
23. , , . *Enterococcus* . 1991;13:229-36.
24. . 1989;21:243-55.
25. , , , , . 1993;11:158-68.
26. . *Streptococcus pneumoniae Enterococcus* . 1993;11:48-55.
27. Murray BE and Mederski-Samoraj B. *Transferable β -lactamase. A new mechanism for in vitro penicillin resistance in Streptococcus faecalis. J Clin Invest* 1983;72:1168-71.
28. , , . *Vancomycin enterococci* . 1998;18:372-8.
29. , , . *Vancomycin* .

1998;18:51-6.