Original Article

Enzymatic chromogenic identification and differentiation of enterococci

[1,2]J. Merlino, [1]G.R. Funnell, [1]D.L. Parkinson, [1]S. Siarakas and [2]D. Veal

[1]Department of Microbiology and Infectious Diseases, Concord Repatriation General Hospital, Concord, NSW
[2]School of Biological Sciences, Macquarie University, Sydney, NSW

ABSTRACT: The application of CHROMagar Orientation (CHROMagar Paris, France) a new chromogenic media containing two chromogenic substrates for the detection of β-D-glucosidase and β-D-galactosidase enzyme activity, together with the L-pyrrolidonyl-β-naphthylamide hydrolysis (PYR) spot test (Murex, Diagnostics), is described for the identification and differentiation of enterococci from other bacterial isolates. In this study, 394 strains of enterococci and 28 strains of streptococci isolated from clinical specimens were cultured on CHROMagar Orientation. After 18 to 24 hours incubation at 35–37°C, all enterococci strains produced a distinctive blue (300C, Pantone Colour Guide chart 747XR 1988–1989) pinpoint-like colony formation and all correlated with results of routine aesculin hydrolysis testing. The 300C blue colour together with a positive PYR test (3 minutes) performed directly from the chromogenic medium allowed presumptive identification of all enterococci tested. Presumptively identified enterococci were confirmed to species level by the use of an algorithm incorporating pyruvate, fermentation of arabinose, and raffinose with optional additional testing of ribose, motility and pigment production as required. Six strains of *Streptococcus bovis* tested were indistinguishable from the enterococcal isolates on CHROMagar Orientation. However, on this medium *S. bovis* produced a negative PYR test. Streptococcus group A, B, C, G, F (n=22) resulted as either pale blue or colourless colonies or did not grow. The colonies were readily distinguishable by colour from other enterococcal and *S. bovis* isolates. CHROMagar Orientation was also found to facilitate easy visual colour detection of pure and mixed cultures of enterococci from direct clinical specimens. Fifty significant positive enterococcal cultures were evaluated and no observable difference was found on growth when compared to 5% horse blood or MacConkey agar. The chromogenic media containing the two chromogenic enzyme substrates demonstrated advantages over the traditional media in presumptively identifying isolates and in detecting mixed growths.

Keywords: CHROMagar Orientation, chromogenic media, β-D-glucosidase, B-D-galactosidase, L-pyrrolidonyl-β-naphthylamide hydrolysis, enterococci, streptococci, identification, differentiation.

Introduction

Enterococci are frequently isolated in clinical laboratories from intra-abdominal infections, urinary tract infections, and bacteremia with or without endocarditis (1-10). Their wide spectrum of isolation in either unimicrobial or polymicrobial infections and their prevalence as a leading cause of nosocomial infections linked to the emergence of vancomycin resistance makes their identification and differentiation from other bacteria important in clinical laboratories (1,3,11-16).

There are a number of commercially prepared selective agars available to most clinical laboratories which allow the isolation and differentiation of enterococci from clinical specimen (17). Most of these plate agar media incorporate azides or antibiotics such as nalidixic acid or phenylethyl alcohol to inhibit the growth of Gram-negative bacteria or other organisms (3,17,18).

The CHROMagar Orientation plate media contains two substrates, β-D-galactopyranoside and β-D-glucopyranoside, linked to different chromogens. Enterococci enzymatically oxidize the β-D-glucopyranoside-chromogen substrate, resulting in a distinctive, pinpoint-like colony, that is blue in colour (300C, Pantone Colour Guide chart 747XR 1988–1989). The β-D-galactopyranoside-chromogen substrate is oxidised by organisms apart from enterococci to give a pink to red colour (19,20-22).

CHROMagar Orientation has the advantage over other agars in that it allows the growth and visual colour detection of enterococci while discriminating and

Address for correspondence:
Mr J. Merlino
Department of Microbiology and Infectious Diseases
Concord Repatriation General Hospital
Hospital Road
Concord NSW 2139

presumptively identifying other organisms that are present in culture. In previous studies it was demonstrated how this medium could be used to presumptively identify not only enterococci, but also other common isolates such as *E. coli* and *Proteus* species (19–21).

This study examines the use of CHROMagar Orientation plate medium and the PYR (L–pyrrolidonyl–β–naphthylamide hydrolysis) spot test (Murex Diagnostics, Australia) together with a reduced range of biochemical fermentation tests for the identification and differentiation of enterococci. This technique may be used to phenotypically identify the most commonly encountered species of enterococci isolated in clinical laboratories. Epidemiologists could use this phenotypic pattern together with other molecular methods such as polymerase chain reaction (PCR)–based or pulsed–field gel electrophoresis (PFGE) to investigate cross–infections or the spread of these organisms in different environments (9,12,13).

Materials and methods

Bacterial isolates

Four hundred and twenty–two clinical isolates recovered by routine means from different specimens were included in this study. The majority were from urine followed by wound, faeces and blood. All bacterial isolates were identified in the Department of Microbiology and Infectious Diseases, Concord Repatriation General Hospital, Sydney, by conventional methods (1,3,4). The enterococci and streptococci tested were all clinical isolates collected in the laboratory within a 12–month period. Each isolate was collected at random. If not tested immediately, the isolate was stored at –70°C until tested. If stored prior to testing, all isolates were sub–cultured at least twice on 5% horse blood agar and all tests were performed on 24 hour cultures. Control strains *Escherichia coli* ATCC35218, *Streptococcus pyogenes* ATCC10389, and *Enterococcus faecalis* ATCC29212, were used to ensure quality control of the media and to assess colour stability.

Culture media

Chromagar media

CHROMagar Orientation (CHROMagar Company, Paris, France) was supplied as a powdered medium by DUTEC Diagnostics, Sydney. The medium contains peptone, meat and yeast extract (16 g/L); agar (15 g/L); and a 'chromogenic mix', at approximately pH 7. The medium was dispensed into petri dishes (17–20 ml into 85–mm dishes). The chromogenic agar was prepared, incubated and plates read according to the manufacturer's instructions, with minor modifications when preparing and reading, as detailed in our previous studies (19–21). Powdered CHROMagar Orientation is added to distilled water and dissolved by slow rotation; when dissolved the medium is boiled under continuous stirring. The medium can also be prepared with a boiling water bath or with flowing steam in an unpressurised autoclave (100°C). Incubation of plates should be in air at 35 to 37°C in the dark. Plates were read after 18 to 24 hours incubation (19).

Enterococccal identification and motility media

Sugar substrates, L (+) arabinose, D (+) raffinose, and pyruvic acid (α–ketopropionic acid) sodium salt were obtained from SIGMA–ALDRICH (Sydney, Australia). Carbohydrate fermentation tests were performed in supplemented nitrogen base media (Mast Lab RM 20) containing 1% of the sugar being tested (4). Motility was determined chromogenically from semi–solid media containing 2,3,5 triphenyltetrazolium chloride (Sigma–Aldrich, Sydney, Australia) (4). Hydrolysis of aesculin was determined on nutrient agar slopes containing 1% aesculin with ferric citrate (4).

Bacterial identification

All isolates were first screened on CHROMagar Orientation for a 300C blue pinpoint–like colour appearance. From the plate medium Gram stains, catalase tests and aesculin hydrolysis were also performed. Isolates were later screened for the fermentation of arabinose, raffinose, and pyruvate using a multipoint inoculation technique. For selected isolates, testing for motility and pigment production (detected by swabbing colonies on horse blood agar following 48–hours incubation) were carried out. A yellow colour on the white cotton swab indicated pigmentation. In addition, other conventional tests were also performed as described by Facklam and Collins (2). API 20 Strep identification systems (BioMerieux, France) was used to confirm identification of certain enterococcal species, especially when acid production from ribose was important. API strips were set up as instructed by the manufacturer. Spot tests Catalase activity was tested by 3% hydrogen peroxide (BDH, AnalaR, Australia) as referenced (3,4,17). The PYR (L–pyrrolidonyl peptidase kit, Murex Diagnostic, Australia), a 3–minute rapid test, was used as described by the manufacturer.

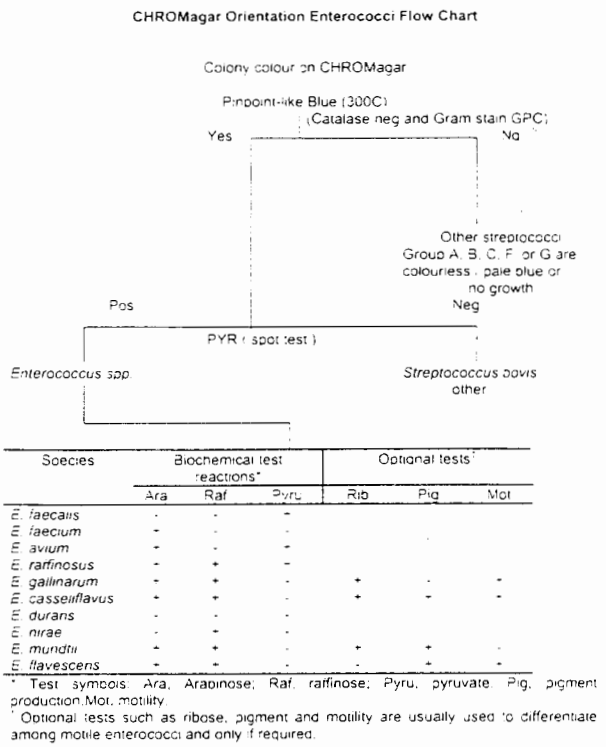
Direct specimen testing

From clinical material received in our hospital, urine specimens from which 40 pure cultures of enterococci and 10 mixed cultures containing enterococci had been isolated were inoculated in parallel on CHROMagar Orientation, 5% horse blood agar, and MacConkey agar after microscopic examination. A 10 μl disposable loop was used. Colistan–nalidixic acid (CNA) plates were also included for heavy mixed growths so that any enterococci would not be missed. Plates were incubated 35 to 37°C for 18 to 24 hours. Growth and colony count were compared on each plate.

Interpretation of results

Interpretation of the colours of colonies on CHROMagar Orientation was as described in our previous studies (19–22). To facilitate the use of this identification algorithm in routine laboratories for the detection of enterococci, a series of flow charts based on our data was created as shown in Figure 1. The pyruvate substrate media contained 0.2% bromothymol blue indicator at pH 7. A change from a negative blue–green to a distinct greenish–yellow indicated positive pyruvate utilisation. The arabinose and raffinose media contained 1.8% phenol red indicator and a change of colony colour from a red–pink to yellow was a positive result indicating that acid had been produced from the sugar substrates. Hydrolysis of aesculin was determined on nutrient agar slopes containing 1% aesculin with ferric citrate. The medium was inoculated by agar stabbing and incubated at 35 to 37°C for 24 hours or longer for up to 7 days. A positive reaction was recorded when the medium turned black. The motility test was interpreted by making a macroscopic examination of the medium for a diffuse zone of growth flaring out from the line of inoculation. The use of tetrazolium salts in this motility

medium helped in the visual detection of bacterial growth. Tetrazolium salts are colourless but are converted into red formazan complexes by the reducing properties of growing bacteria. The development of a red colour helped to trace the spread of bacteria from the inoculation line (17).



Species	Biochemical test reactions*			Optional tests†		
	Ara	Raf	Pyru	Rib	Pig	Mot
E. faecalis	-	-	-			
E. faecium	+	-	-			
E. avium	+	-	+			
E. raffinosus	+	+	-			
E. gallinarum	+	+	-	+	-	+
E. casseliflavus	+	+	-	+	+	+
E. durans	-	-	-			
E. hirae	+	+	-			
E. mundtii	+	+	-	+	+	-
E. flavescens	+	+	-	-	+	+

* Test symbols: Ara, Arabinose; Raf, raffinose; Pyru, pyruvate; Pig, pigment production; Mot, motility.
† Optional tests such as ribose, pigment and motility are usually used to differentiate among motile enterococci and only if required.

Figure 1. Algorithm for identification of common enterococci by means of CHROMagar Orientation and a restricted range of biochemical tests.

Results

Presumptive identification of enterococci

Four hundred and twenty two different isolates were examined for colour production and colony morphology on CHROMagar Orientation as well as PYR reaction and aesculin hydrolysis. All of the enterococcal isolates tested (n=394) in this survey grew on CHROMagar Orientation and displayed a distinctive pinpoint-like blue (300C) colony colour after 18 to 24 hours incubation at 35°C. By combining this growth and colour characteristic together with the rapid PYR spot reaction directly from the CHROMagar Orientation plate medium, all of the enterococcal species in this study could be differentiated from other streptococci to the genus level without the need for further biochemical testing. For all enterococcal isolates tested, the results obtained for CHROMagar Orientation plate medium agreed with the results obtained for aesculin hydrolysis.

Of the *Streptococcus bovis* strains tested (6 of 394), all resulted in a similar colony colour on CHROMagar Orientation. They could not be differentiated on this medium from other enterococcal isolates until a negative PYR spot test was proven. These isolates were characterised further as *S. bovis* by the API 20 Strep identification systems.

On CHROMagar Orientation other streptococci species tested (22 of 394) resulted in either a pale blue or colourless colony, or no growth. No group F streptococci grew on CHROMagar Orientation media. Streptococcus species group A, B and G strains resulted in either colourless or pale light blue colonies. Visually, they could be differentiated from enterococci on CHROMagar Orientation.

Identification of *Enterococcus* species

With the results of a distinctive blue pinpoint-like colour (300C) characteristic on CHROMagar Orientation, a positive PYR test reaction, together with the results of the pyruvate, arabinose, raffinose substrate fermentation, pigment production and motility, all enterococci were identified further to a species level as shown on Table 1. Of the 394 enterococci strains presumptively identified, 339 (85%) were *E. faecalis*, 34 (9%) were *E. faecium*, 7 (2%) were *E. avium*, 6 (2%) were *E. casseliflavus*, 3 (0.8%) were *E. gallinarum*, 3 (0.8%) were *E. raffinosus*, 1 (0.2%) was *E. durans*, and 1 (0.2%) *E. hirae*.

TABLE 1
Biochemical fermentation test results of enterococcal species after 18 to 24 hours incubation at 35 to 37°C

Enterococcus species	Number of isolates tested†	Number of positive test reactions*				
		ARA	RAF	PYU	MOT	PIG
Enterococcus faecalis	339	0	0	339	0	0
Enterococcus faecium	34	34	0	0	0	0
Enterococcus avium	7	7	0	7	0	0
Enterococcus casseliflavus	6	6	6	0	6	6
Enterococcus gallinarum	3	3	3	0	3	0
Enterococcus raffinosus	3	3	3	3	0	0
Enterococcus hirae	1	0	1	0	0	0
Enterococcus durans	1	0	0	0	0	0

* Biochemical tests symbols: ARA – arabinose; RAF – raffinose; PYU – pyruvate; MOT – motility; PIG – pigment (yellow) after 48 hours.
† n – total number of enterococcal isolates tested.

Direct identification and differentiation of enterococci

CHROMagar Orientation permitted good detection of all isolates of enterococci, either in single or mixed bacterial culture as shown on Table 2 and Table 3. No difference was found in colony count of enterococci between CHROMagar Orientation, horse blood, MacConkey, or CNA agars. There was no interference from heavily blood stained or mucoid specimens on colour or growth of enterococcal isolates. In many instances, enterococci were visually presumptively identified from the chromogenic media when compared to horse blood and MacConkey agar. This was more evident with mixed growths, where enterococci are sometimes hidden by the colonies of gram negative bacteria. The chromogenic media gave accurate and reproducible results for direct enterococcal screening.

Discussion

Enterococci have been implicated in many clinical conditions (7,8). Previous studies have demonstrated that enterococci have been associated with nosocomial infections, urinary tract infections, infective endocarditis, and that they are the third most commonly isolated bacteria from intra-abdominal abscesses after *Escherichia coli* and Gram negative anaerobes (6). Studies worldwide indicate that their isolation rate is increasing rapidly (3,15).

TABLE 2
Results of direct urine cultures or pure enterococci on CHROMagar Orientation

Number of specimens tested (n=40)	Microscopy WBC category x 10^6/L	Colour and morphology on CHROMagar Orientation	PYR and GramStain: GPC*	Coloured colony count	Presumptive identification	Traditional testing† and final identification
	>100	Blue pinpoint	Pos		*Enterococcus spp.*	*E. faecalis*
23				>10^8/L		>10^8/L
1				10^7–10^8/L		10^7–10^8/L
1				10^6–10^7/L		10^6–10^7/L
	11–100	Blue pinpoint	Pos		*Enterococcus spp.*	*E. faecalis*
3				>10^8/L		>10^8/L
2				10^7–10^8/L		10^7–10^8/L
	5–10	Blue pinpoint	Pos		*Enterococcus spp.*	*E. faecalis*
1				>10^8/L		>10^8/L
	1–4	Blue pinpoint	Pos		*Enterococcus spp.*	*E. faecalis*
3				>10^8/L		>10^8/L
	<1	Blue pinpoint	Pos		*Enterococcus spp.*	*E. faecalis*
5				>10^8/L		>10^8/L
1				10^6–10^7/L		10^6–10^7/L

* GPC – Gram positive cocci
† Taditional testing included horse blood, MacConkey and CNA agars.

TABLE 3
Results of direct urine cultures of mixed growths on CHROMagar Orientation

Number of specimens tested (n=10)	Microscopy WBC category x 10^6/L	Colour and morphology on CHROMagar Orientation	Coloured colony count	Presumptive identification	Traditional testing and final identification
5	>100	Pink–red / blue pinpoint	>10^8/L	*E. coli* and *Enterococcus spp.*	>10^8/L *E. coli* and *Enterococcus spp.*
1	>100	Clear brown diffused / blue pinpoint	>10^8/L	*Proteus spp.* and *Enterococcus spp.*	>10^8/L *Proteus mirabilis* and *Enterococcus spp.*
1	>100	Large metallic blue / blue pinpoint	>10^8/L	*Klebsiella – Enterobacter* group / *Enterococcus spp.*	>10^8/L *Klebsiella pneumoniae / Enterococcus spp.*
1	11–100	Clear brown diffused / blue pinpoint	>10^8/L	*Proteus spp.* and *Enterococcus spp.*	>10^8/L *Proteus mirabilis* and *Enterococcus spp.*
1	11–100	Clear tinge of green / blue pinpoint	>10^8/L	*Pseudomonas spp.** and *Enterococcus spp.*	>10^8/L *Pseudomonas aeruginosa* and *Enterococcus spp.*
1	11–100	Large metallic blue / blue pinpoint	>10^8/L	*Klebsiella – Enterobacter* group / *Enterococcus spp.*	>10^8/L *Klebsiella pneumonia / Enterococcus spp.*

* Oxidase positive.

Systems that allow rapid and presumptive identification of enterococci to the genus or to the species level are important for the selection of appropriate treatment of certain types of infections such as bacteremia, endocarditis, neonatal infections and infections of the urinary tract and central nervous system. Such systems will also help in obtaining further information on the spread of such microorganisms in our community, especially with the emergence of vancomycin resistant enterococci (VRE) strains as important nosocomial pathogens (1,3,6,13). This point was reinforced by the New South Wales Health Department in September 1996, in circular No. 96/70 (File No. A29855) section 4:

"The microbiology laboratory is the first line of defence against the spread of VRE in hospitals. The laboratory's ability to promptly and accurately identify enterococci and detect vancomycin resistance is essential for recognising VRE colonisation and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed."

Results of this study indicate that the combination of CHROMagar Orientation as a screening agar, combined with the ability to have the PYR test performed directly from this medium, allows the rapid identification and differentiation of enterococci to a genus level. Each isolate of *Enterococcus sp.* tested on this medium

displayed a distinctive blue colour with a pinpoint-like colonial appearance and gave a positive PYR reaction. By combining these two characteristics with the algorithm on Figure 1, all of the commonly encountered enterococci were identified to a species level.

Strains of *S. bovis* tested in this survey also resulted in a pinpoint-like blue colour. The chromogenic medium alone could not differentiate enterococci from *S. bovis*. However, with the addition of a negative PYR reaction, they could be easily categorised as being different, and later identified to species level by other means (24). These findings also correlated with those obtained by the API 20 Strep identification system.

In a previous study, the only other bacterial species to give a similar distinctive blue colour was *Yersinia enterocolitica* (19,20). For this species, colonies were larger than those of enterococci and had a diffuse colour with a distinct 'bulls eye' appearance, i.e., they were quite distinct in colony morphology from enterococci. Other characteristics that can distinguish *Y. enterocolitica* from enterococci and *S. bovis* include a positive catalase test and Gram negative stain morphology.

The distribution of various enterococcal species was similar to those reported in other studies (1,2,7,19,20). The majority of clinical isolates were *E. faecalis* and *E. faecium* (85-90%); other species (*E. avium*, *E. casseliflavus*, *E. gallinarum*, *E. raffinosus*, *E. durans*, and *E. hirae*) accounted for only a small (<10%) percentage of isolates. Since only a small number of isolates belonging to species other than *E. faecalis* were recovered, it must be emphasised that other rare enterococcal species not encountered in our survey such as *E. mundtii*, and *E. flavescens*, can cause infections. They may be recovered from clinical specimens as described by other authors (3,4,25,26). Further studies of those rare organisms on this chromogenic medium must be done before an accurate colony description can be given.

When compared to media such as nalidixic acid agar and phenylethyl alcohol agar, CHROMagar Orientation allows presumptive identification and differentiation of other organisms as well as isolates of enterococci. If used as a screening agar for urinary tract infections or biliary fluids it gives an accurate account and presumptive identification of other bacteria that may be present in mixed culture such as *E. coli* (pink-red) or *Proteus mirabilis* (diffuse-brown). Inhibition of the swarming of *Proteus spp.* on this plate media allows easy visualisation of underlying mixed growth. This makes CHROMagar Orientation an ideal plate medium for the detection of those enterococci frequently encountered in polymicrobial infections associated with the gastrointestinal tract or superficial wounds of hospitalised patients.

Conclusion

This study has shown that by chromogenically targeting β-D-glucosidase enzyme activity of enterococci with CHROMagar Orientation in combination with the PYR spot test and a Gram stain reaction, commonly encountered enterococcal isolates can be presumptively identified to a genus level. When combined with the fermentation of substrates such as arabinose, raffinose, ribose, pyruvate, the production of pigment and motility, the identification of enterococci can be confirmed and they can be easily identified to species level. Small clinical laboratories may find ways to use this scheme to expedite identifications of isolates as an alternative to the setting up of expensive multi-test systems. The study also provides a simple procedure that may benefit investigators, such as medical epidemiologists or clinical microbiologists, in the acquisition of epidemiological data, and an understanding of the spread of enterococci in our community whether in disease or carrier state (9,27).

Acknowledgement

We thank Murex Diagnostics Australia, Dr. Alain Rambach from the CHROMagar Company, Paris and DUTEC Diagnostics for supplying in part materials for this study. Thanks are also due to Mohammad Siddique for medium preparation and the staff of the Department of Microbiology and Infectious Diseases for their excellent technical support. Dr Ross Bradbury and Dr Thomas Gottlieb for their continuing support.

References

1. Bryce EA, Zemcov SJV, Clarke AM. Species identification and antibiotic resistance patterns of enterococci. *Eur J Clin Microbiol Infect Dis* 1991; 10: 745-747.
2. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* 1989; 27: 731-734.
3. Facklam RR, Sahm DF. *Enterococcus*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 6th ed. Washington, DC: ASM Press, 1995; 308-314.
4. Facklam, RR, Washington II JA. *Streptococcus* and related catalase-negative gram-positive cocci. In: Balows A, Hausler WJ, Hermann KL, Isenberg HD, Shadomy HJ. Manual of Clinical Microbiology. 5th ed. Washington, DC: American Society for Microbiology, 1991; 238-256.
5. Felmingham D, Wilson APR, Quintana AI, Gruneberg RN. *Enterococcus* species in urinary tract infections. *Clin Infect Dis* 1992; 15: 295-301.
6. Low DE, Murray BE, Matlow A, Schaberg DR, Shales DM. In: The *Enterococcus* special symposium held in conjunction with the International Congress of Infectious Diseases, Montreal, Canada. Tuesday, July 17, 1990.
7. Moellering RC, Jr. Emergence of *Enterococcus* as a significant pathogen. *Clin Infect Dis* 1992; 1173-1178.
8. Murray BE. The life and times of *Enterococcus*. *Clin Microbiol Rev* 1990; 3:46-65.
9. Joshi N, Milfred D, Caputo G. Vancomycin-resistant enterococci: a review. *Infect Dis Clin Pract* 1996; 5: 528-537.
10. Ruoff KL, De La Mazza L, Murtagh MJ, Spargo JD, Ferraro MJ. Species identities of enterococci isolated from clinical specimens. *J Clin Microbiol* 1990; 28: 435-437.
11. Carbon C, Bichat-Claude B. Contribution of experimental endocarditis to the therapy of multi-resistant enterococci. In: News and Views on Infections. Newsletter of Infectious Disease Council. 1994; Vol. 2, No. 2.
12. Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *J Clin Microbiol* 1993; 37: 2311-2317.
13. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci species. *J Clin Microbiol* 1995; 33: 24-27.
14. Facklam RR, Hollis D, Collins MD. Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. *J Clin Microbiol* 1989; 27: 724-730.
15. Iwen PC, Kelly DM, Linder J, and Hinrichs SH. Revised approach for the identification and detection of ampicillin

and vancomycin resistance in *Enterococcus* species by using microscan panels. *J Clin Microbiol* 1996; 34: 1779–1783.
16. Leclercq R, Courvalin P. Emerging problems with enterococcal infections. *Curr Opin Infect Dis* 1996; 9: 115–119.
17. Koneman EW, Stephen SDA, Dowell VR Jr, Sommers HM. Gram positive cocci. *Enterococcus*–Selective mediums. In: Color atlas and textbook of diagnostic microbiology. Washington. 2nd ed. J.B. Lippincott Company; 1983; 270–271.
18. Devriese LA, Pot B, Van Damme L, Kersters K. Identification of *Enterococcus* species isolated from foods of animal origins. *Int J Food Microbiol* 1995; 26: 187–189.
19. Merlino J, Siarakas S, Robertson GJ, Funnell GR, Gottlieb T, Bradbury R. Evaluation of CHROMagar Orientation for differentiation and presumptive identification of gram–negative bacilli and *Enterococcus* species. *J Clin Microbiol* 1996; 34: 1788–1793.
20. Merlino J, Siarakas S, Robertson GJ, Funnell GR, Gottlieb T, Bradbury R. Abstract. Evaluation of CHROMagar Orientation for differentiation and presumptive identification of gram–negative bacilli and *Enterococcus* species. Abstract. *Aust Microbiol* 1995; 16: 17.3.
21. Merlino J. Application of CHROMagar Orientation in the identification and differentiation of *Proteeae* from other Enterobacteriaceae. *Aust J Med Sci* 1997; 18: 20–23.
22. Merlino J. Coloured colonies. Today's Life Sciences. 1997; 9: 20–24.
23. Wellstood SA. Rapid cost–effective identification of Group A streptococci and enterococci by pyrrolidonyl–β–naphthylamide hydrolysis. *J Clin Microbiol* 1987; 25: 1805–1806.
24. Panosian KJ, Edberg SC. Rapid identification of Streptococcus bovis by using combination enzyme substrate hydrolysis. *J Clin Microbiol* 1989; 27: 1719–1722.
25. Kaufhold A, Ferrieri P. Isolation of *Enterococcus mundtii* from normally sterile sites in two patients. *J Clin Microbiol* 1991; 29: 1075–1077.
26. Verhaegen J, Pattyn P, Hinnekens P, Colaert J. Case reports. Isolation of *Enterococcus avium* from bile and blood in a patient with acute cholecystitis. *J Infect* 1997; 35: 77–78.
27. Donabedian S, Chow JW, Shales DM, Green M, Zervos MJ. DNA hybridization and counter–clamped homogeneous electric field electrophoresis for identification of enterococci to species level. *J Clin Microbiol* 1995; 33: 141–145.