封面式樣

計畫編號：DOH89-TD-1017

行政院衛生署八十八年下半年及八十九年度
科技研究發展計畫

評估台灣地區教學醫院以上偵測萬古黴素腸球菌（vancomycin-resistant enterococci, VRE）之能力（I）

研 究 報 告

執行機構：國防醫學院病理學科

計畫主持人：盧章智

研究人員：

執行期間：88 年 07 月 01 日至 89 年 06 月 30 日

＊＊本研究報告僅供參考，不代表本署意見＊＊

目　錄

頁碼

一、 摘要

1. 中文摘要：

為了使整個計劃「評估台灣地區教學醫院以上偵測萬古黴素抗藥性腸球菌(vancomycin-resistant enterococci, VRE)之能力」執行的好，今年吾人奉核執行衛生署 89 年度(DOH 89-TD-1017)之計劃，在審查委員指示下，吾人於 88.11.21 舉辦「抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研討會」。此次研討會共有 237 人報名，實際參加人數為 214 人，佔報名人數之 90%以上。與會人員有來自地區以上醫院 80 家，其中醫學中心有 8 家，準醫學中心有 6 家，其餘為區域教學醫院以下。研習會為一天課程，吾人邀請了在此領域有相關研究之學者或資深醫檢師來擔任講員。在研習會最後一節，所有與會人員在蔡文城教授主持下定一標準流程來分離及鑑定萬古黴素抗藥性腸球菌。此次研習會最大的收獲乃藉由各專家學者經驗的分享和各醫院檢驗先輩相互的討論，進而建立一致性的鑑定腸球菌屬及 VRE 菌株等步驟。另外與會人員皆感謝衛生署能大力支持此研討會，讓此會能夠順利進行下去。在結束前，研討會人數仍維持在 150 人左右，由與會人員積極參與及其求知慾，可看出台灣大多數醫院檢驗室對 VRE 鑑定需求性極高及對舉辦單位之肯定與鼓勵。結束後，與會人員皆盼望下一次衛生署能繼續支持類似研討會。

中文關鍵詞(至少三個)：萬古黴素抗藥性腸球菌,偵測能力,地區教學醫院

2. 英文摘要：

In order to fulfill the whole project" Evaluation of Ability of Teaching Hospital to Detect Vancomycin-resistant Enterococci in Taiwan", this year we have presented a symposium" Isolation, Detection, Identification of Vancomycin-resistant Enterococci and its Treatment" supported by grant from Department of Health (DOH89-TD-1017) on November 11, 1999. There are 237 preregistrants.

Attendants are 214, it is about 90% of preregistrants. The attendants come from 80 hospitals, including 8 medical center, 6 submedical center, and other local district hospitals. The symposium is one day course. The invited speakers are all specialists or senior technologists who are expert in field of vancomycin-resistant enterococci. By the end of symposium, we have discussed and established a unique method for isolation, detection and identification of VRE. The most benefit in this symposium is that the experts can share their experience and all participants can discuss each other. During this symposium, we established a standard method for identification of enterococci and VRE strains. To let the symposium processing smoothly, the attendants very appreciated DOH that supported this symposium. There are still 150 participants at the end of the symposium. All participants desire to know how to identify VRE because of their intensive participation and desirability. Before closing the symposium, all participants very expect that DOH can support the kind of symposium next time.

Keyword: Vancomycin-Resistant Enterococci, detecting ability, district teaching hospital

二、 本文

1. 前言：

萬古黴素抗藥性腸球菌（vancomycin-resistant enterococci, VRE）為新興的傳染疾病致病源。根據美國國家院感監測系統（Nosocomial Infections Surveillance System）的報告，腸球菌是排名第二的院內感染致病菌及排名第三的院內菌血症致病菌（Schaberg et al., 1991; Lam et al., 1995）。

自從 1986 年分別在法國（Leclercq et al., 1988）及美國（Uttley et al., 1988）分離此菌後，在隨後幾年中，世界各地幾乎都有發現它們的蹤跡（Boyle et al., 1993; Leclercq et al., 1989; Handwerger et al., 1993; Montecalvo et al., 1994）。根據美國疾病管制中心（Centers of Disease Control and Prevention, CDC）所

做的全國性院內感染調查發現（CDC, 1993），在 1989 年時，VRE 僅佔造成院內感染之腸球菌中的 0.3%，到了 1993 年已升高為 7.9%；而在加護病房內 VRE 於 1989 年佔 0.4%，到了 1993 年卻升高為 13.6%。在短短五年之內，VRE 即以二、三十倍的速度成長。在美國這樣一個抗生素管制嚴格的國家都已如此，若相同的問題發生在台灣的話，想必其嚴重性不下於此。並且更令人憂心的是，這些 VRE 的抗藥性基因除了可在腸球菌間彼此傳播外，在實驗室中也已證實可傳遞到葡萄球菌身上（Uttlet et al., 1989; Noble et al., 1992）。Vancomycin 往往是我們拿來對抗具 methicillin 抗藥性之葡萄球菌（methicillin-resistant *Staphylococcus aureus*, MRSA & methicillin-resistant *Staphylococcus epidermidis*, MRSE）的唯一治療藥物，一旦 vancomycin 抗藥性基因傳到 MRSA 或 MRSE 菌株時，其後果將不堪設想，到達無藥可救的地步。目前萬古黴素抗藥性金黃色葡萄球菌（vancomycin-resistant *staphylococcus aureus*, VRSA）在 1997 年六月為日本 Hiramatsu 醫師宣布首次由病患身上分離出後，更讓人憂心忡忡。

在台灣，VRE 菌株於 1995 年被發現後（Ben et al., 1996），隨之，在台灣的幾個醫學中心，如南部的成大醫院、中部的台中 803 總醫院、台北林口長庚醫院、台大醫院及三軍總醫院皆有從臨床檢體上分離出 VRE 菌株之報告（Lu et al., 1997a and 1997b; Perng et al., 1997; Sheu et al., 1998）。在台灣，VRE 菌株通常多半為多抗藥性（multiresistant）之菌株，除了對 vancomycin 有抗藥性外，對於 β-lactams 及 aminoglycosides 藥物也有抗藥情形（Hsueh et al., 1998）。這些菌株常造成臨床醫師治療困擾，特別是當它們突發在加護病房時（Boyce et al., 1992; Karenfil et al., 1992; Livornese et al., 1992），經常引起嚴重感染及致死率。為了要有效的治療及控制其感染，實驗室能提供準確的腸球菌之抗生素敏感試驗是非常重要的，尤其是 vancomycin 之感受性與否。然而偵測 VRE 菌株是有其困難的，特別是 vancomycin 低抗藥性之菌株（Handwerger et al., 1992; Tenover et al., 1993）。

臨床微生物實驗室是防止 VRE 擴散的第一道防線。因此實驗室必須具

備能夠快速且準確之偵測 VRE 菌株的能力。自從 1986 年分離出 VRE 菌株，隔年美國疾病管制中心即開始實施 VRE 監測系統，隨之在北美各洲也有陸續文章探討實驗室對偵測 VRE 菌株之能力（Willey et al., 1992; Tenover et al., 1993; Sader et al., 1994 Dembek et al., 1996; Sahm et al., 1997）。這些研究皆顯示有很多實驗室無法偵測到 VRE 菌株。雖然如此，VRE 菌株之感染所造成醫院及醫師們的衝擊還是非常大，例如統計顯示腸球菌是外科傷口感染及院內泌尿系統感染之第二位致病原，是引起菌血症之第三位致病原（Schaberg et al., 1991）。

在國內，1995 年 VRE 出現至今已有四年了。雖然它曾造成人的死亡，也曾在加護病房引起地區性的流行（Lu et al., 1996a），國立陽明大學蔡文城教授也曾於前年（87）三月舉辦重要院感病原菌之偵測和快速鑑定及藥敏試驗之研習會，但據吾人所知，VRE 在台灣成長速度或嚴重情形並不像美國。吾人無法知道此現象是因為事實如此或是我們偵測及鑑定 VRE 菌株能力有問題以致於低估了它。在此困擾下，因而引發本研究的構想，希望能了解台灣地區教學醫院以上對 VRE 分離的能力及分離情形，盼望藉此計劃能幫助衛生署建立 VRE 之監測系統，建立本土 VRE 之基本資料以輔助臨床醫師能有效控制此 VRE 感染。

2. 材科與方法

本計劃為乃藉由評估台灣地區教學醫院以上對 VRE 菌株之偵測能力及藥物敏感試驗之測試以及相關的研習討論會，希望能進一步的建立 VRE 之診斷及監測系統之研究計劃。今年計劃主要目的乃舉辦 VRE 分離、鑑定及抗生素敏感性試驗之研討會（研討會內容如附件一）。首先吾人發邀請函及報名表至各相關醫院（如附件二），在這同時吾人向臨床病理醫學會及醫事檢驗學會申請學分證明以便讓參予學員有學分證書，然後於民國八十八年十一月二十一日舉辦「抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會」。

3. 結果與討論

此次研習會共有 237 人報名，實際參加人數為 214 人，佔報名人數之 90% 以上，其簽到名冊如附件三。研習會為一天課程，吾人邀請了在此領域有相關研究之學者或資深醫檢師來擔任講員，其講義如附件一。與會人員有來自地區以上醫院約 80 家，其中醫學中心有 8 家，準醫學中心有 6 家，其餘為區域教學醫院以下。與會人員於早上八時一開始就非常積極參與聽講及討論，在休息時間雖有茶點招待，也很熱絡與講員討論相關問題。下什三時半休息時間為第二次簽到時間，與會學員簽到後，才分別發予臨床病理醫學會或醫事檢驗學會各八學分證書，如附件四。在研習會最後一節，所有與會人員在蔡文城教授主持下定一標準流程來分離及鑑定萬古黴素抗藥性腸球菌，如附件五。

4. 結論與建議

此次研習會最大的收獲乃藉由各專家學者經驗的分享和各醫院檢驗先輩相互的討論，進而建立一致性的鑑定腸球菌屬及 VRE 菌株等步驟。另外與會人員皆感謝衛生署能大力支持此研討會，讓此會能夠順利進行下去。在結束前，研討會人數仍維持在 150 人左右，由與會人員積極參與及其求知慾，可看出台灣大多數醫院檢驗室對 VRE 鑑定需求性極高及對舉辦單位之肯定與鼓勵。結束後，與會人員皆盼望下一次衛生署能繼續支持類似研討會。

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附件(二)

[redacted]

緣起：萬古黴素腸球菌（vancomycin-resistant enterococci, VRE）菌株於 1995 年在台灣出現，至今已四年了。據吾人所知，VRE 在台灣的成長速度並不如美國那麼嚴重，吾人無法知道此現象是事實或是由於台灣在偵測及鑑定 VRE 菌株方面的能力尚不足，以致於低估了其嚴重性。此次研習會乃由衛生署主辦，三軍總醫院承辦，希望藉由此研習會的形式讓台灣相關之臨床微生物實驗室能夠普遍了解 VRE 菌株之特性，進一步建立一套標準分離與鑑定 VRE 菌株之方法，且有能力準確地偵測 VRE 菌株。竭誠歡迎大家踴躍參與，共襄盛舉。

對象：(1) 請各級醫院之臨床微生物或細菌組務必派一至二人參加。
(2) 醫院各相關醫師及醫檢師同仁們。
(3) 有興趣之醫界、學界人員。

主辦單位：行政院衛生署

承辦單位：三軍總醫院臨床病理科

協辦單位：中華民國臨床病理學會（７個小時學分證書正在申請中）
中華民國醫事檢驗學會

舉辦地點：三軍總醫院醫療大樓二樓電化教室

主持人：盧章智副教授兼主任醫師

時間：88 年 11 月 20 日（星期六）08:30～17:00

招生學員人數：暫定 100 人

費用：免費，提供午餐及茶點

聯絡電話：(02) 2368-0235　　傳真號碼：(02) 2368-0235

聯絡人：盧章智醫師或戴美娟小姐

報名方式：請填妥報名表寄回（１００）台北市汀州路三段八號三軍總醫院臨床病理科或傳真至 (02) 2368-0235。

截止日期：1999 年 11 月 10 日（星期三），請早日報名以免向隅。

敬　請　公　佈

內容：

11 月 20 日（星期六）

08:00～08:30　報到

08:30～09:20　傳統鑑定腸球菌屬之經驗談

（林口長庚醫院 臨床理病科 技術部主任　吳竹蘭）

09:20～10:10　VRE 菌株之分離與鑑定經驗談

（三軍總醫院 臨床理病科 醫檢師　黃麗文）

10:10～10:30　休息

10:30～11:20　從直腸拭子篩選 VRE 菌株之經驗談

（三軍總醫院 臨床病理科 助理教授兼細菌組組長　陳繼祥）

11:20～12:10　VRE 感染之臨床表現與治療

（台大醫院 檢驗醫學部 主治醫師　薛博仁）

12:10～13:30　午餐

13:30～14:20　VRE 之快速鑑定

（三軍總醫院 臨床病理科 醫檢師兼講師　彭成立）

14:20～15:10　探討台灣 VRE 菌株之特性

（三軍總醫院 臨床病理科 副教授兼主任醫師　盧章智）

15:10～15:30　休息

15:30～16:20　VRE 院感調查經驗談

（三軍總醫院 小兒科 副教授兼主任醫師　王志堅）

16:20～17:10　討論並建立統一分析 VRE 鑑定方法

(陽明大學 微免學科 教授　蔡文城)

✂----------

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會報名表

姓名：_____　性別：_____　籍貫：_____

出生日期：_____年_____月_____日

連絡電話：_____　傳真號碼：_____

通訊地址：_____

_____○住宅　○服務單位

職務名稱：_____醫師　_____醫檢師　_____研究者　_____其他

服務機關單位：_____

三軍總醫院抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研討會主辦單位(函)

正本：全國各大醫院　　中華民國八十八年九月二十日

主旨：為了讓台灣相關之臨床微生物實驗室能夠普遍了解萬古黴素腸球菌（vancomycin-resistant enterococci, VRE）菌株之特性，進而建立一套標準分離與鑑定 VRE 菌株之方法，及能正確地偵測 VRE 菌株。本研究室將承辦此次研討會，邀請國內相關學者分享其經驗及心得，敬請派員參加並給與公差為荷。

說明：1. 本研討會乃由衛生署之計劃「建立快速偵測萬古黴素腸球菌之技術」（計劃編號：DOH 88-TD-1005）所提供的。

2. 萬古黴素腸球菌菌株於 1995 年在台灣出現，至今已四年，此菌株在台灣的成長速度並不如美國那麼嚴重，研究者無法確知此現象真是事實或是由於台灣在偵測及鑑定 VRE 菌株方面的能力尚不足，以致於低估了其嚴重性。因此，本研究室將承辦台灣北中南部抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研討會，藉以提昇台灣相關之臨床微生物實驗室在這方面的能力。

3. 研討會將頒發 7 小時之繼續教育學分證書給予受訓學員，並贈送研討會之講義。

4. 研討會報名截止日期為 11 月 4 日。

5. 檢附有關研討會講員、題目、時間、地點等有關資料乙份，敬請參考，並踴躍選派相關人員參加且給與公差為荷。

研討會主持人

盧章智

附件(三)

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會

服務單位	姓名	簽名		備註
		上午	下午	
中山附設醫院 (1)	吳秀鴻			
台北市新光醫院 (2)	王雅瓔	王雅瓔	王雅瓔	88002
	戴筱芸	戴筱芸	戴筱芸	005
	粘峰銘	粘峰銘	粘峰銘	006
台北醫學院附設醫院 (3)	林鴻圖			
台大醫院 (4)	陳君竹	陳君竹	陳君竹	88001
	顏麗琴	顏麗琴	顏麗琴	88003
	林秀英	林秀英	林秀英	004
	杜心慧	杜心慧	杜心慧	005
	陳萍弘	陳萍弘	陳萍弘	006
	陳玉姬	陳玉姬	陳玉姬	007
	陳美伶	陳美伶	陳美伶	008
	潘惠如	潘惠如	潘惠如	009
	謝德淳	謝德淳	謝德淳	010
台中榮民總醫院 (5)	石建民	石建民	石建民	011
	張靜玟	張靜玟	張靜玟	012
	朱雅玫 改	朱雅玫	朱雅玫	~~015~~ 008
行政院衛生署台中醫院 (6)	王燕珠			
	花世民	花世民	花世民	013
台北中山醫院 (7)	施佩君	施佩君	施佩君	014

服務單位	姓名	簽名		備註
		上午	下午	
台北中山醫院 (8)	徐淑惠	徐淑惠	徐淑惠	016
	劉鎮寧	劉鎮寧	劉鎮寧	017
	林芝芬	林芝芬	林芝芬	018
	黃惠玲	黃惠玲	黃惠玲	019
台北市仁愛醫院 (9)	譚可瑩	譚可瑩	譚可瑩	020
	魏秋芳	魏秋芳	魏秋芳	021
	謝瑛璋	謝瑛璋	謝瑛璋	022
	劉圓鏡	劉圓鏡	劉圓鏡	023
台北市仁愛醫院 (10)	鄭素華	鄭素華	鄭素華	024
台北市立性病防治所(11)	張安隆	張安隆	張安隆	025
台北長庚醫院 (12)	莊千慧	莊千慧	莊千慧	026
	張志光	張志光	張志光	027
	王少玲			
台北馬偕醫院 (13)	黃淑慈	黃淑慈	黃淑慈	028
台北榮總 (14)	黃湘君	黃湘君	黃湘君	029
	邱豐弟養	邱豐弟養	邱豐弟養	030
	劉湘萍	劉湘萍	劉湘萍	031
	林美伶	林美伶	林美伶	032
	林玉真	林玉真	林玉真	033
	朱淑君	朱淑君	朱淑君	034

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會

服務單位	姓名	簽名		備註
		上午	下午	
台北醫院 (15)	吳沁曼			036
	吳心怡			036
台安醫院 (16)	李怡穎	李怡穎	李怡穎	037 35
	簡鳳如	簡凰如	簡凰如	038 36
	吳麗娟	吳麗娟	吳麗娟	039 37
	賴雨辰			040
	劉蕙蘭	劉蕙蘭	劉蕙蘭	041 38
	林美琦			042
	林春美			043
	陳美智	陳美智	陳美智	044 39
	劉成傳			045
	陳思樺	陳思樺	陳思樺	046 40
	陳怡君	陳怡君	陳怡君	047 41
	黃淑萍	黃淑萍	黃淑萍	048 42
	李岩	李光玉	李光玉	049 43
	柳鐏鈺	柳鐏鈺	柳鐏鈺	050 44
台南市立醫院 (17)	李昭儀			051
台北市立和平醫院 (18)	黃怡彰	黃怡彰	黃怡彰	052
	吳宜蘭	吳宜蘭	吳宜蘭	045 45
台北市立陽明醫院	饒永珍			

附件(四)

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會學分證明

編號：

茲證明　　君

於民國八十八年十一月二十日參加由行政院衛生署及三軍總醫院臨床病理科舉辦：

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會

經中華民國臨床病理醫學會及中華民國醫事檢驗學會認可各發予 **八小時** 學分

主辦單位：行政院衛生署
承辦單位：三軍總醫院臨床病理科
協辦單位：中華民國臨床病理醫學會
　　中華民國醫事檢驗學會

三軍總醫院臨床病理科

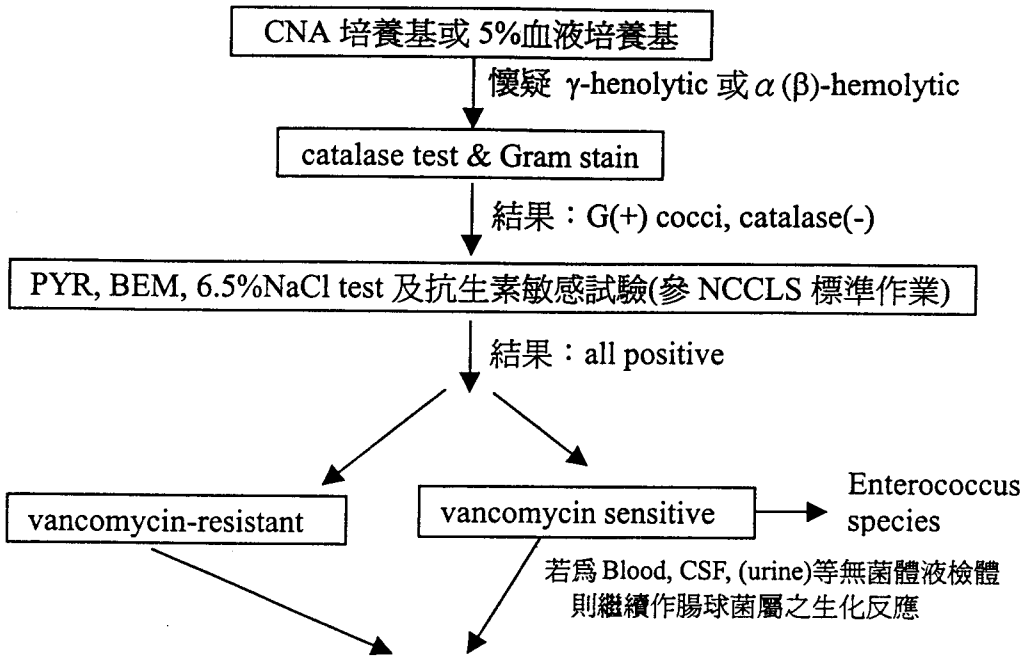
抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會籌備委員會

主辦人：盧章智

中華民國八十八年十一月二十日

附件(五)

腸球菌屬之鑑定與抗生素敏感試驗流程圖

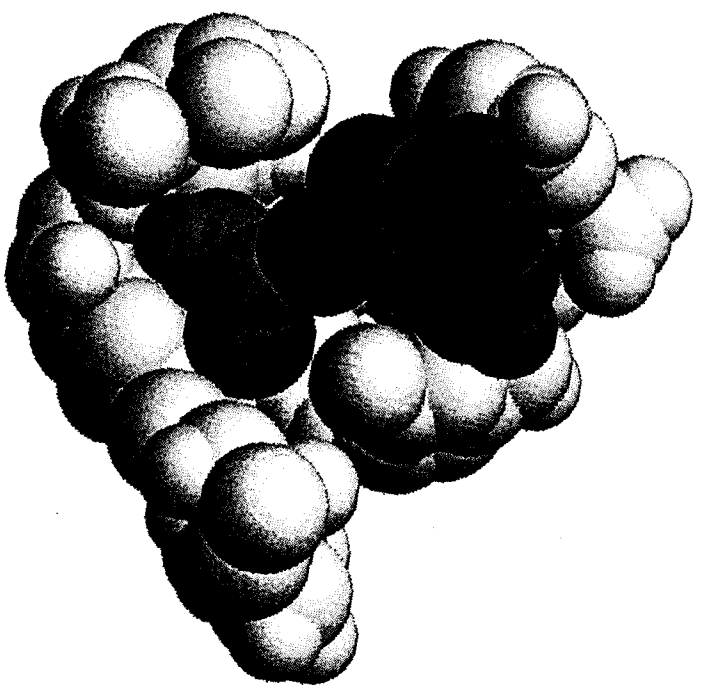


腸球菌屬之生化反應

Species	MAN	SOR	ARG	ARA	SBL	MOT	PIG	PYU	MGP
Group I									
E. avium	+	+	−	+	+	−	−	+	+
E. malodoratus	+	+	−	−	+	−	−	+	−
E. raffinosus	+	+	−	+	+	−	−	+	+
E. pseudoavium	+	+	−	−	+	−	−	+	+
E. saccharolyticus	+	+	−	−	+	−	−	−	+
Group II									
E. faecalis	+*	−	+*	−	+	−	−	+	−[b]
Lactococcus spp.	+	−	+	−	−	−	−	−	−
E. faecium	+*	−	+	+	v	−	−	−	−
E. casseliflavus	+	−	+*	+	v	+*	+*	v	+
E. mundtii	+	−	+	+	v	−	+	−	−
E. gallinarum	+*	−	+*	+	−	+*	−	−	+
Group III									
E. durans	−	−	+	−	−	−	−	−	−
E. hirae	−	−	+	−	−	−	−	−	−
E. dispar	−	−	+	−	−	−	−	+	+
Group IV									
E. sulfureus	−	−	−	−	−	−	+	−	+
E. cecorum	−	−	−	−	+	−	−	+	−
Group V									
E. columbae	+	−	−	+	+	−	−	+	−
V. fluvialis	+	−	−	−	+	+	−	−	+

MAN, mannitol; SOR, sorbose; ARG, arginine; ARA, arabinose; SBL, sorbitol; MOT, 0.04% tellurite; MOT, motility; PIG, pigment; PYU, pyruvate; MGP, methyl-α-D-glucopyranoside; EFRO, efrotomycin disk (100 μg); +, >90%; -, <10% positive; v, variable; +* or -*, occasional exceptions (<3% of strains show aberrant reaction).

抗萬古黴素腸球菌之分離、偵測、鑑定與治療之研習會



時間：中華民國八十八年十一月二十日

主辦單位：行政院衛生署

承辦單位：三軍總醫院臨床病理科

協辦單位：中國民國臨床病理醫學會

中華民國醫事檢驗學會

目錄

附件一

VRE研習會成果目錄

傳統鑑定腸球菌屬的經驗談

吳竹蘭

長庚紀念醫院林口醫學中心
臨床病理科

傳統鑑定腸球菌屬的經驗談

吳竹蘭
長庚紀念醫院林口醫學中心
臨床病理科

Enterococcus

- Streptococcus faecalis and S. faecium
- 1970 Kalina Proposed Enterococcus spp.based on cellular arrangement and phenotypic characteristics
- 1984 Schleifer and Kilipper-Balz

Enterococcus

- 17 species
- Gram positive (oval) cocci, occurs singly,in pairs, in short chain
- Facultatively anaerobic, optimum growth temp. 37 °C, growth range 10-45 °C
- Catalase (-), pseudocatalase in E. faecalis
- Gr.D antigen (+)

Enterococcus Infection

- Urinary Tract Infection
- Intra-Abdominal or Pelvic wound infection
- Bacteremia

Isolation of Enterococcus in CGMH, 7-12, 1998

Species	Total	Urine	Ascites	Wound	Blood
E. faecalis	1221(80%)	301	54	588	78
E. faecium	50 (3.3%)	12	5	13	7
E. avium	39	2	7	13	2
E. gallinarum/E. faecium	4	1			2
E. casseliflavus	4	1			6
E. pseudoavium	3			2	
E. raffinosus	13		2	4	1
E. hirae	1	1			
E. durans	19	6	1	2	4
Enterococcus spp.	167 (11%)	26	7	58	8

Isolation

- BAP
- CNA or PEA

Identification-Between catalase negative group

- Hemolysis
- Smear
- Vancomycin susceptibility
- LAP
- PYR
- Bile esculin
- 6.5% NaCl
- Growth at 10 and 45 °C

genus	Cell Arrangement	Van	Gas	PYR	LAP	BE	NaCl	10°C	45°C	Hem
Enterococcus	chain	S	-	+	+	+	+	+	+	$\alpha\beta\gamma$
Streptococcus	chain	S	-	-*	+	-*	-*	-	V	$\alpha\beta\gamma$
Lactococcus	chain	S	-	+	+	+	V	+	-	$\alpha\gamma$
Leuconostoc	chain	R	+	-	-	V	V	+	V	$\alpha\gamma$
Pediococcus	Chain, tetracoccus	R	-	-	+	+	V	-	+	α
Aerococcus	Chain, tetracoccus	S	-	+	-	V	+	-	+	α
Gemella	Chain, tetracoccus	S	-	+	V	-	-	-	-	$\alpha\gamma$
Helcococcus	Chain, tetracoccus	S	-	+	-	+	+	-	-	γ

Vancomycin susceptibility

- 5-10 colonies
- TSA plate
- 30 μg Vacomycin paper disk
- 35°C, CO_2 Incubator, overnight
- Any zone as " S "

Bile esculin

- 40% Bile
- 1% Esculin
- 35°C, CO_2 Incubator
- overnight and 48 hours reading
- Black colony

6.5% NaCl

- Heart Infusion agar with 6.5% NaCl, 0.1% Glucose
- BCP as indicator
- 35°C, up to 72 hours
- any growth

PYR

- Pyrrolidonyl arylamidase
- Substrate-L-pyroglutamic acid -β-naphthylamide and p-dimethylaminocinnaldehyde
- bright pink
- Commercial available rapid test

LAP

- Leucine aminopeptidase
- Rapid Strep identification system (Analytab products)

Growth temperature check

- Heart Infusion broth with 0.1% glucose
- BTB as indicator
- Single colony or a drop of broth culture
- 10°C or 45°C up to 7 days
- frank growth as positive

Conventional Identification Method for Enterococcus species

- **Mannitol, Sorbose, Arabinose, Sorbitol, Raffinose, Sucrose, (Ribose)**
- **Pyruvate utilization**
- **Arginine**
- **0.04% Tellurite**
- **Motility**
- **Pigment**
- **methyl α-D-Glucopyranoside**
- **Efrotomycin susceptibility**

Carbohydrate fermentation

- Mannitol, Sorbose, Arabinose, Sorbitol, Raffinose, Sucrose, MGP (methyl α-D-Glucopyranoside)
- Heart infusion broth with 1% carbohydrate
- BCP as indicator
- 35°C, up to 7 days
- Positive -change to yellow color

Pyruvate utilization

- Na Pyruvate 1%, BTB as indicator
- 35°C, up to 7 days
- Positive : change to yellow color

0.04% Tellurite

- Heart infusion agar with blood and Potassium tellurite
- 35°C, up to 7 days
- Blcak colony

Arginine

- Moller decarboxylase medium with 1% arginine
- BCP as indiccator
- 35°C, up to 7 days
- Pisitive: change to purple color

Efrotomycin susceptibility

- BAP
- EFRP paper disk (1 mg)
- 35°C, overnight
- a growth inhibition of any size as " S "

Modified Conventional Identification Method

Species	ARG	SBL	ARA	RAF	Pigment	
E. faecalis	+	+	-	-		
E. faecium	+	v	+	v		++++ +-+- +++- +-++
E. gallinarum	+	-	+	+		
E. casseliflavus	+	v	+	+	+	+-++ ++++
E. avium	-	+	+	-		
E. pseudoavium	-	+	-	-		
E. raffinosus	-	+	+	+		
E. hirae	+	-	-	V		+--+ +---
E. durans	+	-	-	-		

Comparison between 1day and 7 days reading

Species	7 day results	1 days results	Difference
E. faecalis	8	8	
E. faecium	9	9	
E. avium	6	5	Arabinose
E. gallinarum/E. faecium	6	6	
E. casseliflavus	4	2	Arginine
E. pseudoavium	2	2	
E. raffinosus	2	2	
E. hirae	2	0	Raffinose
E. durans	8	8	
E. species(++-+)	1	1	
E. species(--+-)	4	4	
Total	52	47(90.4%)	

Commercial System-rapid ID 32 strep

- bioMérieux sa
- ADH, β GLU, βGAR, βGUR,α GAL,PAL,APPA, βGAL, PryA, βNAG, GTA, βMAN, URE
- RIB, MAN, COR, LAC, TRE, RAF, SAC, LARA, DARA, CDEX, GLYG, PUL, MAL, MEL, MLZ, MBDG, TAG
- VP, HIP

Commercial System-rapid ID 32 strep

- Inoculum: 4 McFarland
- 37°C, 4 hours

BBL CRYSTAL identification systems-Gram positive ID Kit

- α-D-glucoside, L pyroglutamic acid, L-trp, ARG, β-D-glucoside, L-val, L-phe, N acetyl β D glucosaminide, phosphate,β D glucuronide, L- Ile, , β D cellobioside, PLN, α D maltoside, ONPG
- TRE, LAC, MAB, SUC, MAN, Maltotriose, ARA, Glycerol, FRC, BGL,
- URE, ESC

BBL CRYSTAL identification systems-Gram positive ID Kit

- Inoculum: 0.5 McFarland
- 35-37°C, 24 hours

Comparison among Conventional method, API32C and Crystal system

Species	7 day results	1 days results	Crystal	ATB 32C
E. faecalis(6)	4 (E. durans)	4 (E.durans)	6	6
E. faecium(9)	6 (3 E.gallinarum/ E. faecium))	6 (3 E.gallinarum/ E.faecium)	9	9
E. avium(3)	3	3	2(E.raffinosus)	2(Lactococcus)
E. casseliflavus(4)	4	2 (E. spp)	2(Helcococcus)	4
E. raffinosus(2)	2	2	2	0 (1 E. avium) (1 E. faecium)
E. hirae(2)	2	0(E. durans)	0(E. durans)	2
E. durans(1)	1	1	1	1
E. species(4)	3 (E. pseudoavium)	3 (E. pseudoavium)	0	0

VRE 菌株之分離與鑑定

黃麗文

三軍總醫院臨床病理科
細菌組

VRE 菌株之分離與鑑定

三軍總醫院臨床病理科
細菌組 黃麗文

Enterococci

- The second most common cause of *nosocominal infection*
- Vancomycin-resistant enterococcus *cross-infection*

Recovery of Vancomycin-Resistant Gram-positive Cocci from Pediatric Liver Transplant Recipients

- 49 patients
- MIC ≥8 μg/ml , considered resistant to vancomycin
- Infection due to VRE developed in 3 patients, a urinary tract infection in two and peritonitis in one.
- E. faecium

- Stool colonization with VRE may be common and early finding among pediatric liver transplant recipients
- Infection appear to be uncommon

Isolation of VRE

- 1. Clinical infection
  Blood, Urine, Wound, Tip...
- 2. Colonization
  Rectal swab

Screen Medium

- CNA blood agar plate with vancomycin
- Campylobacter blood agar plate with vancomycin
- Columbia CNA-Va 30 μg disc blood agar plate
- Enterococcosel broth with vancomycin
- Enterococcosel agar with vancomycin

Vancomycin resistance screening agar

1.Brain heart infusion agar with 6 μg/ml of vancomycin

2.E. casseliflavus and E. gallinarum
could grow, regardless of the MIC of vancomycin

3.E. faecium
only MIC ≥ 4 μg/ml could grow

Evaluation of Vitek and API 20S

- The percentage of correctly identified organism was (Vitek/API)
  - 97.6% / 79.2% (E. faecalis)
  - 95.3% / 91.2% (E. faecium)
  - 86.0% / 20.7% (Non-faecalis -non-faecium species)
- Predictive value
  - 98.1% for Vitek, 85.9% for API

API 20 strep

- Has been shown to be useful in the identification of gram positive isolates.
- Previous reports have suggested that this systems perform well for identifying the most common species such as E. faecalis and E. faecium, but the identification of non-faecalis-non-faecium species does not appear to optimal.

- The most common API 20S errors was the identification of E. faecalis as E. faecium.
- Misidentified 20% of E. faecalis and 9% of E. faecium isolates

Motility and pigment production test together with commercial test system are sufficient for reliable identification of E. faecium, E. gallinarum and E. casseliflavus

Typical and Atypical stains

E. casseliflavus : motility ,non-motility
pigment , non-pigment
E. gallinarium : motility , non-motility

Acidification of Methyl-α-D-Glucopyranoside

- To differentiate E. casselifkavus and E. gallinarum from E. faecium and E. faecalis
- Acid production

- Methyl-α-D-Glucopyranoside was dissolved in dist. water, filter sterilized
- Phenol red broth base
- Conc. 1%
- Distributed in 2 ml
- 37 °C 24-48 hours
- Positive : yellow color
  Negative : red color

- Methyl-α-D-Glucopyranoside is a necessary component of routine VRE screerning

Species	Result for			
	motility	pigment	MGP	EFRO
E. faecalis	-	-	-	R
E. faecium	-	-	-	S
E. casseliflavus	+·	+·	+	R
E. mundtii	-	+	-	S
E. gallinarium	+·	-	+	R

- MGP: Methyl-α-D-Glucopyranoside
- EFRO: Efrotomycin

Simple and Rapid Methods for Identifying E.faecalis, E. faecium, E. casseliflavus, E. gallinaurm

- Litmus milk
- Arabinose
- Rapid motility
- MGP

Organism	% positive by			
	LM	ARA	RM	MGP
E. faecalis	100	0	0	0
E. faecium	0	100	0	0
E. casseliflavus	96	92	96	100
E. gallinarum	100	90	100	95

Selective and differentiate broth

- Enterococcosel broth
  with vancomycin 8 μg/ml
- 35°C , 24-48 hours
- Black and turbid

Vancomycin Resistant Enterococcus

Disk diffusion test :

Va (30 μg) disc zone size: ≤14 mm(S)
15-16 mm(I)
≥17 mm(R)

E-test :

MIC value: ≤ 4 μg/ml (S)
8-16 μg/ml (I)
≥32 μg /ml(R)

Vancomycin Resistant

- Enterococcus
- Leuconostoc
- Erysipelothrix
- Lactobacillus
- Pediococcus

Gram stain

Gram positive cocci:
Enterococcus
Leuconostoc
Pediococcus

Gram positive rod:
Lactobacillus
Erysipelothrix1

BEM, PYR

Genus	BEM	PYR
Enterococcus	+	+
Leuconostoc	V	-
Pediococcus	+-	-

Enterococcus Group II

	Mannitol	Arginine	Sorbose
E. faecalis	+	+	-
E. faceium	+	+	-
E. casseliflavus	+	+	-
E. mundtii	+	+	-
E. flavescens	+	+	-
E. gallinarum	+	+	-

	Pigment	Ribose	Arabinose	Motility	Pyrruvate	M-α-d-g
E. faecalis	-	+	-	-	+	-
E. faceium	-	+	+	-	-	-
E. casseliflavus	+	+	+	+	-	+
E. mundtii	+	+	+	-	-	-
E. flavescens	+	-	+	+	-	+
E. gallinarum	-	+	+	+	-	+

從直腸拭子篩選 VRE 菌株之經驗談

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Selective Media for Detecting Fecal Carriage of VRE

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Nosocomial Infections Surveillance System

- Entercocci are the second most common cause of surgical wound infections, and nosocomial urinary tract infection
- The third most common cause of nosocomial bacteremias

Uttley

- November 1986, 55 strains of VRE derived from twenty-two patients with end-stage renal failure or multiple organ failure
- Sources include blood, intra-abdominal sepsis, urine, peritoneal fluid, pleural fluid, and bile
- 48 strains of E. faecium and 7 were E faecalis and some strains had vancomycin MIC greater than 2000 μg/ml (Europe)

Sahm

- February 1987, three VRE strains derived from 2 patients in blood culture
- Three strains were E. faecalis and had vancomycin MICs ranged from 32 to 64 μg/ml (U.S.A)

Nosocomial Infections Surveillance System

- Vancomycin-resistant enterococci(VRE) have emerged as an important cause of hospital acquired infection
- From 1989 to1993, the proportion of nosocomial infections that cause by VRE had increased from 0.3 to 7.9 %

Tenover (1995)

- Many vancomycin resistant of enterococci (VRE) are resistant to β-lactams, aminoglycosides, fluoroquinolones, and other antibiotics

VRE

- Colonization
- Clinical infection

Henning(1996)

- In the majority of cases, invasive and colonizing isolates were identical by DNA fingerprinting techniques,suggesting that the colonizing VRE was the source of infection
- Intermittent excretion of organisms in stool makes vigilant tracking and immediate isolation of such patients crucial to control effort

Normal Intestinal Flora

- S. epidermidis, Diphtheroides, Enterococci, Streptococci, rare S, aureus, and yeasts
- Enterobacteriaceae: lactose fermenter and lactose non-fermenter
- Non-fermentative gram-negative bacilli

VRE

- Antibiotics:vancomycin, aztreonam, kanamycin, cefaperazone, colistin, nalidixic acid, amphotericin B
- Chemicals:sodium azide, oxolinic acid

VRE

- Colistin-nalidixic acid blood agar
  vacomycin 5 μg/ml, 8 μg/ml, 10 μg/ml
  amphotericin B 8 μg/ml
- Campylobacter blood agar
  vacomycin 10 μg/ml
  cefaperazone 20 μg/ml

VRE

- Blood agar
  vacomycin 4 μg/ml
  amphotericin B 2 μg/ml
  aztreonam 4 μg/ml
- Enterococcosel agar
  vancomycin 6 μg/ml ,8 μg/ml ,10 μg/ml
- Enterococcosel broth
  vancomycin 6 μg/ml ,8 μg/ml ,10 μg/ml

Ford (1994)

- Cephalexin-aztreonam-arabinose agar
  arabinose fermenter:Yellow(surrounding medium)
  arabinose non-fermenter :red (surrounding medium)
  Yellow:E. faecium
  Red:E. faecalis

Jordens (1994)

- Kanamycin-aesculin-azide (KAA) broth
  sodium azide 0.15 μg/ml
  vancomycin 20 μg/ml

Audicana (1995)

- Oxolinic acid-aesculim-azide(OAA)agar
  sodium azide 0.4 μg/ml
  oxolinic acid 5 μg/ml
-

Coque (1996)

- Streptococcus faecalis agar
  vancomycin 6 μg/ml
- Streptococcus faecalis broth
  vancomycin 6 μg/ml

Merlino (1996)

- Chromoagar orientation
  E. faecalis:blue color and pinpoint colony
  E. faecium: blue color and pinpoint colony

Clinical Specimens

- One thousand and five hundred rectal swabs, from January to June,1999
- Six hundred and forty nine strains of VRE

Media

- CNA-VA (8 μg/ml) blood agar
- Campylobacter blood agar
- Columbia CNA-VA 30 μg disc blood agar
- Enterococcosel broth-VA (8 μg/ml)
- Enterococcosel agar-VA (8 μg/ml)

MIC of Vancomycin

- E-test (AB Biodisk)
- Incubation 35 °C, 24 h
- MIC : ellipse of growth inhibition intersect E-test strip

Disc diffusion test

- Mueller-Hinton agar II
- Vancomycin, penicillin-G , ampicillin erythromycin, gentamicin-h, amoxillin/ clavulanic acid
- Incubation 35 °C, 24 h

Summary

- EBVA: VRE≥8 μg/ml all growth
- EAVA: VRE≥8 μg/ml all growth
- CNA-VA:
  VRE≥8 μg/ml all growth
  P. aeruginosa growth
- CAMP:
  VRE≥8 μg/ml all growth
  P. aeruginosa growth

Summary

- EBVA: 649, 97.5 %
- EAVA: 547, 82.1 %
- CNA-VA: 188, 28.2 %
- CNA-VAD: 177, 26.6 %
- CAMP: 103, 15.5 %

Summary

- Mixed strains
  EBVA: 32 strains
  EAVA: 16 strains
  CNA-VA: 5 strains
  CNA-VAD: 1 strain
  CAMP: 1 strain

Summary

- E. faecium: all resistant, with exception of chloramphenicol and teicoplanin
- E. faecalis: ampicillin, penicillin-G, and Amoxicillin/clavulanic acid are sensitive
- E. gallinarum: ampicillin, penicillin-G, and Amoxicillin/clavulanic acid are sensitive

Summary

- Klare (1995)
  With the use of selective media, low numbers of organisms might be missed

Summary

- Sahm (1997)
  E. faecium, Van A or Van B, zone size>6 mm
  E. gallinarum or E. casseliflavus, Van C, zone size< 15 mm

Summary

- Aderson (1997)
  EAVA: sensitive, rapid
  EBVA: over sensitive, slow
  In EBVA, the growth of various organisms with vancomycin MIC value of less than 6 μg/ml

NCCLs

- Resistance: ≥32 μg/ml , <14 mm
- Intermediate: 8-16 μg/ml, 15-16 mm
- Susceptible: <4 μg/ml, ≥17 mm

Summary

- Vancomycin MIC 8 μg/ml versus 6 μg/ml
- Stool colonization, to many intrinsic strains of E. gallinarum
- Increase work load
- No clinical significance

VRE 感染之臨床表現與治療

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EMERGENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCI AT A UNIVERSITY HOSPITAL IN TAIWAN: PERSISTENCE OF MULTIPLE SPECIES AND MULTIPLE CLONES

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OBJECTIVES: To describe the epidemiology of vancomycin-resistant enterococci (VRE) in a university hospital in Taipei, Taiwan.
DESIGN: Retrospective review over a 27-month period, from March 1996 to May 1998.
SETTING: National Taiwan University Hospital (NTUH), a tertiary-care teaching hospital in Taiwan.
PARTICIPANTS: Patients with VRE isolated from any body site.
METHODS: Patients were identified through hospital microbiology and infection control records. Patient charts were reviewed for clinical and epidemiology data, including age, gender, previous hospital admissions, underlying diseases, types of infection, and recent antibiotic use. VRE isolates were characterized by their typical biochemical reactions, cellular fatty acid profiles, and the presence of *van* genes. Antibiotypes using the E test and random amplified polymorphic DNA (RAPD) patterns of these isolates were used to determine the clonality.
RESULTS: Twenty-five isolates of VRE recovered from 12 patients were identified. One patient with a perianal abscess had 12 isolates of VRE (four isolates of *Enterococcus faecalis*, seven of *E. faecium*, and one of *E. casseliflavus*) recovered from perianal lesions. Among three patients who were hospitalized in the same room, one had a community-acquired cellulitis over the left leg caused by *E. faecalis* and the other two patients both had anal colonization with two isolates of *E faecalis*. The other eight patients had one *E. faecalis* isolate each from various clinical specimens. All isolates possessed *vanA* resistance phenotype and *vanA* genes. Multiple species of VRE (*E. faecalis, E. faecium.* and *E. casseliflavus*) and multiple clones of *E. faecium* could colonize in and/or infect hospitalized patients. In addition, same clones of VRE can persist long-term in patients' lower gastrointestinal tract. Different antibiotypes and RAPD patterns of the isolates from different patients excluded the possibility of nosocomial spread at the hospital.
CONCLUSIONS: These results extend our knowledge of the coexistence and the persistence of multiple species and multiple clones of VRE in hospitalized patients.

(Infect Control Hosp Epidemiol, Dec. 1999)

ANTIMICROBIAL SUSCEPTIBILITIES OF CLINICAL ISOLATES OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN TAIWAN

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Abstract: To understand the antimicrobial resistance patterns of vancomycin-resistant enterococci in Taiwan, we tested the *in vitro* activities of 10 antimicrobial agents against 71 clinical isolates (39 of *Enterococcus faecalis* and 32 of *Enterococcus faecium*) by means of the agar dilution method. Resistance was determined on the basis of the minimum inhibitory concentration (MIC) of each antimicrobial agent—MIC_{50} and MIC_{90} (minimum concentrations required to inhibit growth of 50% and 90% of isolates, respectively) were determined. No β-lactamase producers were identified with the cefinase test. All *E. faecalis* isolates were susceptible to penicillin and ampicillin, and 97% of these isolates were resistant to teicoplanin (*vanA* phenotype). Of the *E. faecium* isolates, 75% were susceptible to teicoplanin (*vanB* phenotype) and most were resistant to penicillin (94%) and ampicillin (94%). Quinupristin/dalfopristin was markedly less active against *E. faecalis* than *E. faecium* isolates (MIC_{50}, 64 vs 2 μg/mL; MIC_{90}, 128 vs 8 μg/mL; susceptibility rates, 3% vs 81%). Five of the eight *vanA* phenotype *E. faecium* isolates and one of the 24 *vanB* phenotype *E. faecium* isolates were resistant to quinupristin/dalfopristin. The activity of rifampin was also species-specific, with *E. faecium* being markedly less susceptible to this agent than *E. faecalis* (MIC_{50}, 16 vs 1 μg/mL; MIC_{90}, 64 vs 4 μg/mL). Our data suggest the potential of teicoplanin and quinupristin/dalfopristin as appropriate antimicrobial agents in the treatment of infections caused by *vanB* phenotype *E. faecium*. Penicillin, ampicillin, and rifampin alone, or preferably in combination with other agents, appear to be the most appropriate agents for the treatment of vancomycin-resistant *E. faecalis* infections in Taiwan.

(J Formos Med Assoc 1999;98:45–8)

Key words:
vancomycin-resistant enterococci
antimicrobial susceptibility

ıncomycin-resistant enterococci (VRE) have been in-ɛasing in prevalence as major nosocomial pathogens in ıny countries since their first description in 1988 [1–3]. :ording to the National Nosocomial Infection Surveil-ıce System in the USA, the percentage of nosocomial terococcal pathogens that were resistant to vancomy-ı increased 26-fold from 1989 (0.3%) to 1993 (7.9%)]. These organisms have intrinsic resistance to many timicrobial agents, notably penicillinase-resistant nicillins, cephalosporins, aminoglycosides, and ndamycin. Moreover, the prevalence of acquired resistance to penicillin and high-level resistance to aminoglycosides has been increasing in recent years [1, 2, 5]. With the addition of vancomycin resistance to this profile, some of these organisms are virtually untreatable with currently available antimicrobial agents [1, 2, 5–7]. Recently, several new agents including a semisynthetic streptogramin (quinupristin/dalfopristin) and a glycopeptide derivative (LY333328) have shown good *in vitro* activity against VRE [8–11]. However, their roles for clinical use in the treatment of serious infections caused by these organisms are still under investigation [2].

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In Taiwan, the first clinical strain of vancomycin-resistant *E. faecalis* was reported in 1996 [12]. Since then, several reports have documented increasing rates of isolation of VRE, in both colonization and invasive infections in hospitalized patients [13, 14]. As the prevalence of VRE in nosocomial infections increases, it is important to determine the *in vitro* activities of old and new antimicrobial agents against these organisms. In this study, we determined antimicrobial susceptibilities of 71 clinical isolates of VRE to 10 agents, which is essential for understanding the therapeutic options for treatment of infections caused by these organisms in Taiwan.

Materials and Methods

From December 1996 to July 1998, a total of 71 isolates of VRE (39 *E. faecalis* and 32 *E. faecium*) were recovered from various clinical specimens (Table 1) of patients treated at three major teaching hospitals in Taiwan: National Taiwan University Hospital, Taipei (30 isolates), National Cheng-Kung University Hospital, Tainan (28 isolates), and Tri-Service General Hospital, Taipei (13 isolates). Specimens of rectal swabs were inoculated onto vancomycin screening agar (brain heart infusion agar with 6 μg/mL of vancomycin) [3]. All the isolates were characterized by standard microbiologic methods, including hydrolysis of esculin and growth in 6.5% NaCl. Resistance to vancomycin was evaluated with the disk diffusion method [3]. The organisms were identified to the species level with the following two commercial identification systems: the API 20 Strep system (BioMerieux Vitek, Hazelwood, MO, USA) and the API 32 Strep system (BioMerieux Vitek). All isolates were stored at −70°C in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 15% glycerol and were subcultured onto trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems) at least twice before antimicrobial susceptibility testing. The β-lactamase activity of the isolates was determined by means of a chromogenic cephalosporin assay (Cefinase; BBL Microbiology Systems).

Minimum inhibitory concentrations (MICs) of the 71 isolates of VRE were determined using the agar dilution method with Mueller-Hinton agar (BBL Microbiology Systems) according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [15]. Antimicrobial agents tested in this study were obtained from the manufacturers as standard powders for laboratory use: penicillin, ampicillin, gentamicin, rifampin, sulbactam, and vancomycin from Sigma Chemicals (St. Louis, MO, USA); imipenem from Merck Sharp & Dohme (West Point, PA, USA); ciprofloxacin from Bayer (Leverkusen, Germany); teicoplanin from Marion-Merrell Dow (Cincinnati, OH, USA); and quinupristin/dalfopristin from Rhone-Poulenc Rorer (Collegeville, PA, USA). The concentrations of these drugs ranged from 0.03 to 256 μg/mL, except for gentamicin, which ranged from 0.03 to 1,024 μg/mL. The isolates were incubated at 35°C in ambient air, and the MIC results were read after 24 hours' incubation for vancomycin and after 18 hours' incubation for other agents. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as control strains in each set of tests.

MIC breakpoints for defining susceptibility were as described by the NCCLS [15]. High-level resistance to gentamicin was defined as an MIC of above 500 μg/mL. Isolates with vancomycin MICs of at least 32 μg/mL were considered resistant. Three vancomycin-resistant phenotypes were categorized: *vanA* phenotype (vancomycin MIC ≥ 64 μg/mL and teicoplanin MIC ≥ 16 μg/mL), *vanB* phenotype (vancomycin MIC 16–512 μg/mL and teicoplanin MIC ≤ 8 μg/mL), and *vanC* phenotype (vancomycin MIC 2–32 μg/mL; teicoplanin MIC ≤ 8 μg/mL) [3]. If the highest tested concentration did not inhibit growth, the isolate was considered to have an MIC of at least 512 μg/mL, or 2,052 μg/mL for gentamicin. The MIC_{50} and MIC_{90} (minimum concentrations required to inhibit growth of 50% and 90% of isolates, respectively) of the antimicrobial agents were also calculated.

Table 1. Sources of 71 isolates of vancomycin-resistant enterococci

Specimen	No. of isolates	
	Enterococcus faecalis	*Enterococcus faecium*
Blood	5	2
Ascites	2	0
Wound (pus)	13	7
Urine	3	5
Sputum	2	0
Drainage fluid	4	4
Rectal swab	9	13
Stool	1	1
Total	39	32

Results

Table 2 shows the *in vitro* susceptibility results of the 71 VRE isolates to the 10 antimicrobial agents tested.

Table 2. *In vitro* susceptibilities of 71 vancomycin-resistant enterococci isolates to 10 antimicrobial agents

Organism (No.)/ Antimicrobial agent	MIC (μg/mL)*			Susceptible isolates	
	Range	MIC_{50}	MIC_{90}	Breakpoint (μg/mL)	%
Enterococcus faecalis (39)					
Penicillin	1–8	2	4	≤ 8	100
Ampicillin	0.25–16	0.5	4	≤ 8	97
Ampicillin/sulbactam	0.25–16	0.5	2	NP	NP
Imipenem	1–8	2	4	NP	NP
Ciprofloxacin	2–128	4	64	≤ 1	0
Rifampin	0.5–8	1	4	NP	NP
Gentamicin	4–≥ 2,052	16	≥ 2,052	NP	NP
Vancomycin	≥ 512	≥ 512	≥ 512	≤ 4	0
Teicoplanin	16–≥ 512	128	≥ 512	≤ 8	0
Quinupristin/dalfopristin	4–≥ 512	64	128	≤ 4	3
Enterococcus faecium (32)					
Penicillin	1–≥ 512	≥ 512	≥ 512	≤ 8	6
Ampicillin	1–≥ 512	128	≥ 512	≤ 8	6
Ampicillin/sulbactam	1–≥ 512	128	≥ 512	NP	NP
Imipenem	2–≥ 512	128	≥ 512	NP	NP
Ciprofloxacin	2–≥ 512	64	128	≤ 1	0
Rifampin	0.03–≥ 512	16	64	NP	NP
Gentamicin	8–≥ 2,052	≥ 2,052	≥ 2,052	NP	NP
Vancomycin	128–≥ 512	≥ 512	≥ 512	≤ 4	0
Teicoplanin	1–≥ 512	2	≥ 512	≤ 8	75
Quinupristin/dalfopristin	0.5–16	2	8	£ 4	81

*Concentrations required to inhibit visible growth of 50% and 90% of clinical isolates, respectively. NP = antimicrobial agents for which the interpretive criteria for susceptibility of *Enterococcus* species are not provided by the National Committee for Clinical Laboratory Standards (NCCLS).

None of the isolates tested were positive for β-lactamase by the cefinase disk test. All *E. faecalis* and eight *E. faecium* isolates demonstrated *vanA* phenotypes. The remaining 24 *E. faecium* isolates had *vanB* phenotypes.

Quinupristin/dalfopristin had good activity against most isolates of *E. faecium*. Twenty-six (81%) isolates were inhibited at a concentration of 4 mg/mL or less, making it the most active of the drugs tested. However, quinupristin/dalfopristin was markedly less active against *E. faecalis* than *E. faecium* isolates (Table 2). Of the eight *E. faecium* isolates with the *vanA* phenotype, five were also resistant to quinupristin/dalfopristin. Of the remaining 24 *E. faecium* isolates with the *vanB* phenotype, only one was resistant to quinupristin/dalfopristin.

Susceptibilities to penicillin, ampicillin, ampicillin/sulbactam, imipenem, and rifampin were also species-specific. *E. faecium* isolates were markedly more resistant to penicillin, ampicillin, and ampicillin/sulbactam than *E. faecalis* isolates. The MIC_{50} of imipenem and rifampin for *E. faecium* isolates was 16- to 64-fold higher than for *E. faecalis* isolates, while the MIC_{90} of these two drugs for *E. faecium* isolates was 16- to 128-fold higher than those for *E. faecalis* isolates. Twenty-five of the *E. faecalis* isolates were inhibited by rifampin at a concentration of 1 μg/mL. All isolates were resistant to ciprofloxacin.

High-level resistance to gentamicin was found in 13 *E. faecalis* isolates and 27 *E. faecium* isolates, with the MICs for these resistant isolates being at least 2,052 μg/mL in all cases.

Discussion

Several prior studies have reported quinupristin/dalfopristin to be active both *in vitro* and clinically for infections caused by a broad range of gram-positive organisms, including vancomycin-susceptible and resistant *E. faecium* [6–10]. These studies also demonstrated that the concentration of this agent required for inhibition of enterococci is 0.25 to 32 μg/mL [6, 7, 12]. Quinupristin/dalfopristin has been shown to be inactive against *E. faecalis* [6, 7, 10]. Our results were partly consistent with these findings.

Compared with the susceptibility results reported by other investigators [6–10], quinupristin/dalfopristin had lower activity against the enterococcal isolates in

our study. The MIC_{50} and MIC_{90} of quinupristin/dalfopristin were four- to eight-fold higher for our *E. faecium* isolates, and 16-fold higher for *E. faecalis* isolates, compared with previous studies [6–10]. In addition, our study showed that the activity of this agent against the *E. faecium* isolates with the *vanA* phenotype was markedly poorer than against the *vanB* phenotype isolates. At present, quinupristin/dalfopristin has not yet been introduced in Taiwan. Given the increasing MICs of quinupristin/dalfopristin among vancomycin-resistant *E. faecium* isolates, continuous monitoring of the *in vitro* activity of this agent against *E. faecium* is necessary to determine whether or not it is an appropriate therapeutic option for treatment of infection due to this organism.

E. faecium has been demonstrated to be more resistant than *E. faecalis* to penicillins, imipenem, and other β-lactam antibiotics [8, 10]. Ciprofloxacin and gentamicin have both been shown to have poor activity against these two species [6–8]. Our results support these observations. Interestingly, we found that the activity of rifampin was also species-specific, with *E. faecium* tending to be less susceptible than *E. faecalis*. Livornese et al reported successful treatment of two patients with vancomycin-resistant *E. faecium* bacteremia using a combination of ciprofloxacin, rifampin, and gentamicin [16]. They also demonstrated the *in vitro* synergy of this regimen, although Bonilla et al showed a lack of synergy when quinupristin/dalfopristin was combined with rifampin for treating *E. faecium* infections [8]. There have been anecdotal reports of successes when rifampin was used in combination with other active agents for treating bacteremia caused by vancomycin-resistant *E. faecium* [2, 8, 17]. Our data suggest that rifampin, preferably in combination with other agents, might be a feasible alternative therapy for VRE in Taiwan, particularly for *E. faecalis*. However, further clinical evaluations are needed to confirm the effectiveness of rifampin.

In summary, the *in vitro* susceptibility data of the limited number of Taiwanese isolates of VRE suggest the potential of teicoplanin and quinupristin/dalfopristin as therapeutic agents for the treatment of vancomycin-resistant *E. faecium* infections. Penicillin, ampicillin, and rifampin, alone or preferably in combination with other agents, according to the susceptibility results, are suitable for treating vancomycin-resistant *E. faecalis* infections in Taiwan.

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13. Perng CL, Lu JJ, Chi WM: Characterization of vancomycin-resistant enterococci isolated in northern Taiwan. In: *Proceedings of the Annual Meeting of the Chinese Society of Microbiology, 1997, Taipei, Taiwan.* Taipei: Chinese Society of Microbiology, 1997. [Abstract]
14. Sheu SM, Huang AH, Wu JJ: Characterization of vancomycin-resistant enterococci in southern Taiwan. In: *Proceedings of the Annual Meeting of the Chinese Society of Microbiology 1997, Taipei, Taiwan.* Taipei: Chinese Society of Microbiology, 1997. [Abstract]
15. National Committee for Clinical Laboratory Standards. *Performance Standard for Antimicrobial Susceptibility Testing: Eighth Informational Supplement*, NCCLS Document M100-S8. Villanova, PA: National Committee for Clinical Laboratory Standards, 1998.
16. Livornese LL, Dias S, Samel C, et al: Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* by electronic thermometer. *Ann Intern Med* 1992;117: 112–6.
17. Feldman RJ, Paul SM, Silber JL: An analysis of treatment of patients with vancomycin-resistant enterococcal bacteremia. *Infect Dis Clin Prac* 1996;5: 440–5.

VRE 之快速鑑定

彭成立

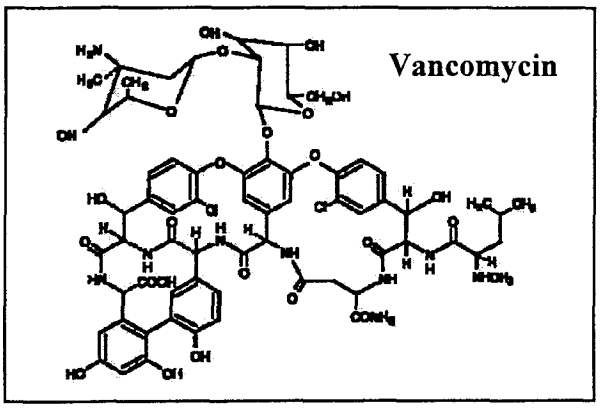
三軍總醫院臨床病理科
分子診斷實驗室

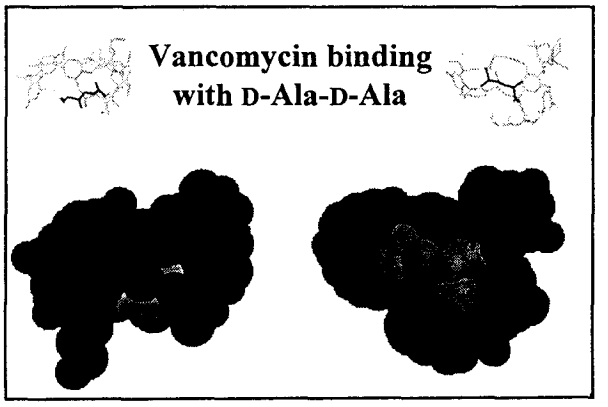
VRE之快速鑑定

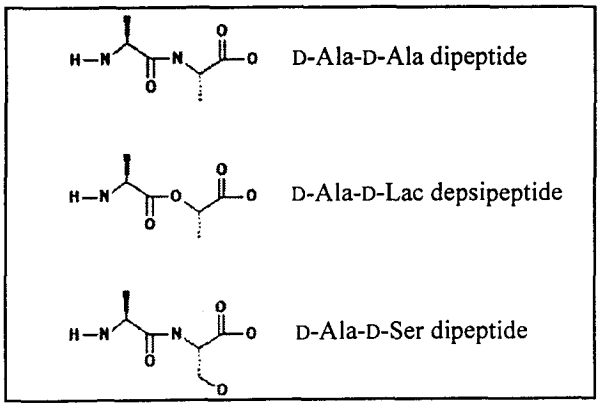
三軍總醫院 臨床病理科
分子診斷實驗室
彭成立

Introduction (I)

- What is VRE ?
  - Vancomycin Resistant Enterococci
- What is vancomycin ?
  - Glycopeptide antibiotic
  - Structure
- What is the mechanism of vancomycin resistance ?
  - D-Ala-D-X ligase
  - Change D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser



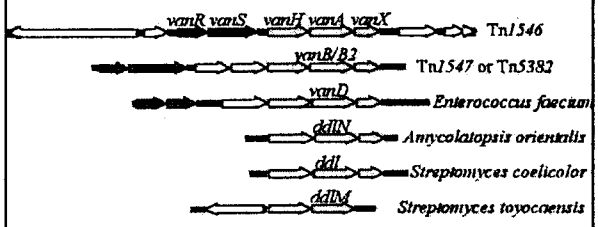




Introduction (II)

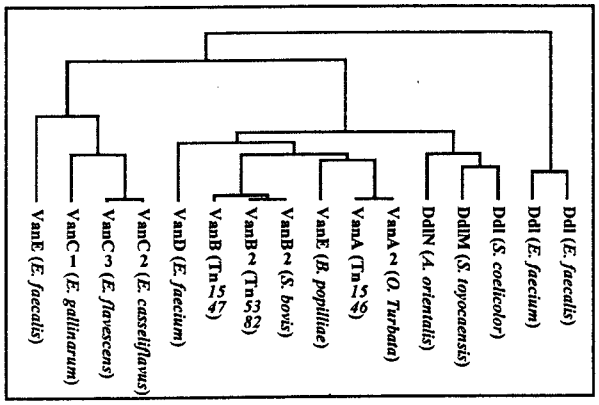
- How many types are there of vancomycin resistant genes?
  - ***vanA***
  - ***vanB, vanB2***
  - ***vanC1, vanC2, vanC3***
  - ***vanD***
  - ***vanE***

Introduction (III)



Introduction (IV)

vanA2	*Oerskovia turbata*
vanB2	*Streptococcus bovis*
vanC1	*Enterococcus gallinarum*
vanC2	*Enterococcus casseliflavus*
vanC3	*Enterococcus flavescens*
vanE	*Enterococcus faecalis*
vanE	*Bacillus popilliae*
ddl	*Enterococcus faecalis*
ddl	*Enterococcus faecium*



Introduction (V)

- Rapid and accurate identification of two most serious phenotypes of VRE, VanA and VanB, is critical for appropriate management of the treatment of infections and prevention of their spread.
- VanA phenotype displays high level resistance to vancomycin (MIC > 64 µg/ml) and teicoplanin and is controlled by the vanA gene. Detection of *vanA* is of particular concern because of its potential transfer to other gram-positive bacteria.

Introduction (VI)

- VanB phenotype displays low to high level resistance to vancomycin (MIC from 8 to > 64 µg/ml) and susceptibility to teicoplanin. Identification of this phenotype is difficult due to its variable level of resistance. Both *vanB* and *vanB2* genes are associated with the VanB phenotype.
- VanC phenotype is characterized by low level resistance to vancomycin (MIC 8 - 32 µg/ml) and susceptibility to teicoplanin. VanC is not considered clinically significant.
- Conventional susceptibility tests for VRE identification are time consuming (> 24 hours) and cannot reliably identify low level resistant phenotypes.

The methods for detection and identification of VRE

- Phenotypic scheme
  - conventional method
  - screen broth or agar
    - bile esculin azide with 6 µg of vancomycin
- Genomic scheme (molecular techniques)
  - Probe hybridization
  - Nucleic acid amplification

Molecular detection of resistance genes

- Hybridization
  - conventional DNA probe
    - radioisotope
    - enzyme
    - antigenic tag
    - fluorescence
    - acridinium ester
  - Q-beta replicase based probe amplification
  - Branched DNA (b-DNA) based signal amplification
  - Cycling probe technology
    - DNA-RNA-DNA probe

Molecular detection of resistance genes

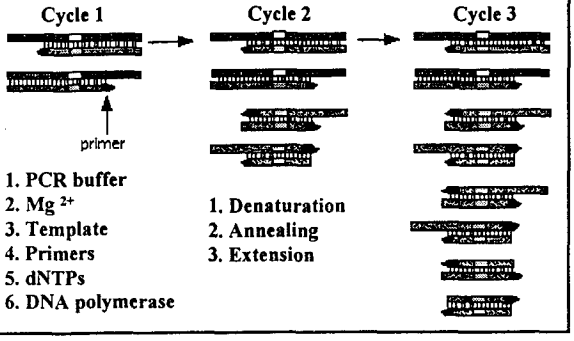
- Nucleic acid amplification
  - Polymerase chain reaction (PCR)
    - Single primer pair PCR
    - Multiplex PCR
  - Ligase chain reaction (LCR)
  - Strand displacement amplification (SDA)
  - Transcription based amplification system (TAS)
    - Self-sustaining sequence replication (3SR)

Published papers for detection of VRE by different molecular techniques

•PCR	74
•CPT	1
•Qβ	0
•LCR	0
•SDA	0
•TAS/3SA	0

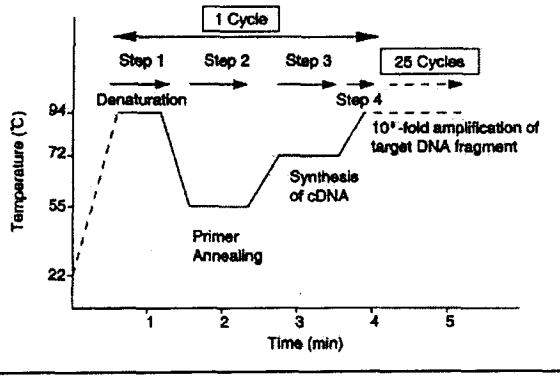
Polymerase Chain Reaction

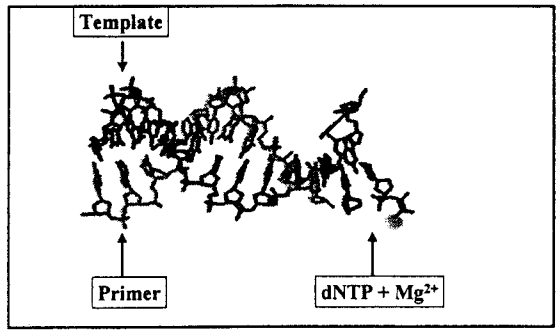
Polymerase Chain Reaction



1. PCR buffer
2. Mg^{2+}
3. Template
4. Primers
5. dNTPs
6. DNA polymerase

1. Denaturation
2. Annealing
3. Extension





Taq DNA polymerase

Different methods of nucleic acid isolation for PCR

- No preparation
- Freeze-thaw then boiling
- Boiling in distilled water, TE Triton, PCR buffer
- Lysozyme + SDS + phenol/$CHCl_3$ + ethanol ppt.
- Commercial kit
  - Chaotrope silica method
    - QIAGEN QIAamp tissue kit
  - 5% Chelex-100
- Mechanical disruption (minibeadbeater)
- Sonication

Detection methods for PCR products

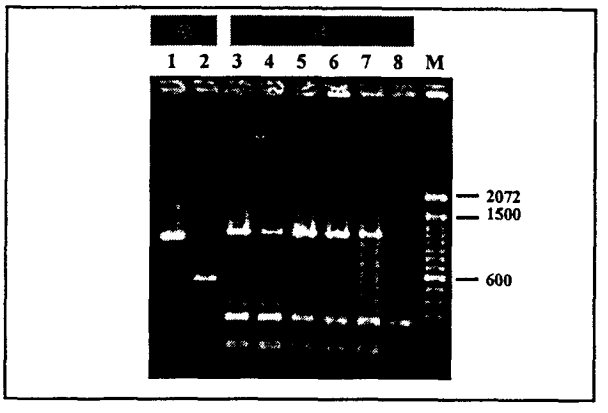
- Gel electrophoresis + Ethidium bromide staining
  - Agarose
  - Polyacrylamide gel electrophoresis (PAGE)
- Gel electrophoresis + probe hybridization
- Restriction fragment length polymorphism (RFLP) + Electrophoresis + EtBr
- Sandwich hybridization
- Real time detection by fluorescence
  - Energy transfer or quenching
  - Fluorescence polarization

PCR (I)

Characterization of Glycopeptide-Resistant Enterococci from U.S. Hospitals

Clark NC, *et al.* Antimicrob. Agents Chemother. 1993. 37:2311-7.

Gene	Primers(5'->3')	Product (bp)
vanA	CAT GAA TAG AAT AAA AGT TGC AAT A CCC CTT TAA CGC TAA TAC GAT CAA	1030
vanB	GTG ACA AAC CGG AGG CGA GGA CCG CCA TCC TCC TGC AAA AAA	433
vanC	GAA AGA CAA CAG GAA GAC CGC ATC GCA TCA CAA GCA CCA ATC	796

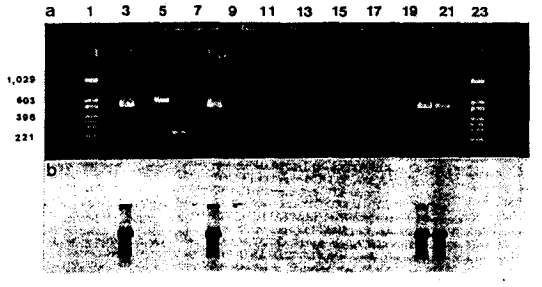


PCR (II)

Vancomycin-Resistant Enterococci at a Large University Hospital in Denmark

Kjerulf A, *et al.* APMIS 1996. 104:475-9.

Gel electrophoresis
Southern hybridization by *vanA* probe



PCR (III)

Detection of Enterococcal Vancomycin Resistance by Multiplex PCR

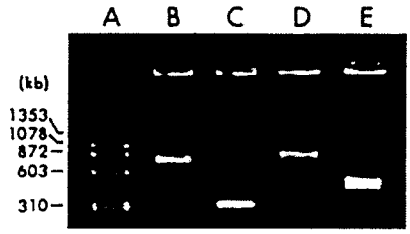
Free L, *et al.* PCR protocols for emerging infectious diseases. ASM Press, Washington, D. C. 1996. p. 150-6.

Gene	Primers(5'->3')	Product(bp)
vanA	GCT ATT CAG CTG TAC TC CAG CGG CCA TCA TAC GG	783
vanB	CAT CGC CGT CCC CGA ATT TCA AA GAT GCG GAA GAT ACC GTG GCT	297
vanC1	GGT ATC AAG GAA ACC TC CTT CCG CCA TCA TAG CT	822
vanC2	CTC CTA CGA TTC TCT TG CGA GCA AGA CCT TTA AG	439

PCR mixture		
A	10 x Buffer	10.00
	$MgCl_2$ (25mM)	20.00
	dNTP (2.5mM)	8.00
	VanA1(25p/μL)	2.00
	VanA2(25p/μL)	2.00
	VanB1(25p/μL)	2.00
	VanB2(25p/μL)	2.00
	H_2O	48.50
B	*Taq* polymerase(5U/μL)	0.50
C	DNA solution	5.00

PCR cycle condition		
95°C	3 min	1 cycle
94°C 56°C 72°C	1 min 1 min 1 min	30 cycles
72°C 4°C	7 min hold	1 cycle

Multiplex-PCR

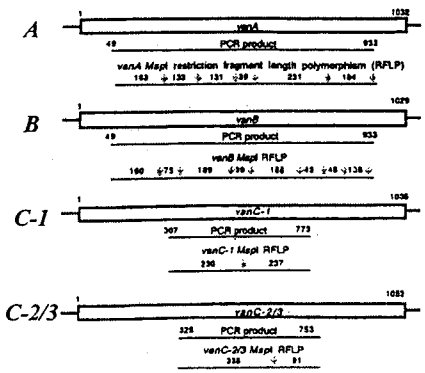


PCR (IV)

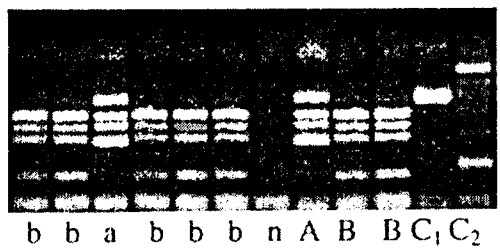
Multiplex PCR Detection of *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* Genes in Enterococci

Patel R, *et al.* J. Clin. Microbiol. 1997. 35:703-7.

Gene	Primers(5'->3')	Product(bp)
vanA	CAT GAC GTA TCG GTA AAA TC ACC GGG CAG RGT ATT GAC	885
vanB	CAT GAT GTG TCG GTA AAA TC ACC GGG CAG RGT ATT GAC	885
vanC1	GAT GGC WGT ATC CAA GGA GTG ATC GTG GCG CTG	467
vanC2/3	GAT GGC WGT ATC CAA GGA ATC GAA AAA GCC GTC TAC	429



M-PCR + RFLP by *Msp* I

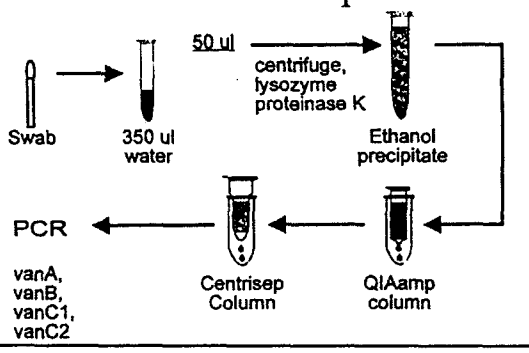


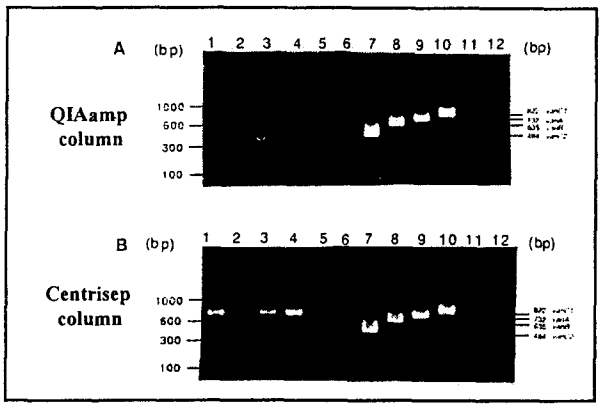
PCR (V)

Detection of Vancomycin-Resistant Enterococci in Fecal Samples by PCR

Satake S, *et al.* J. Clin. Microbiol. 1997. 35:2325-30.

From fecal samples





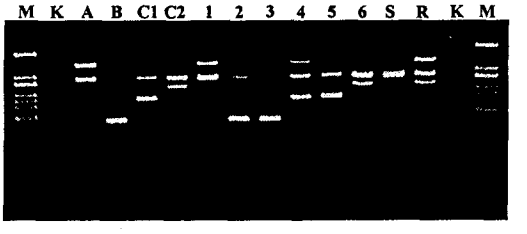
PCR (VI)

Detection of Clinical Vancomycin Resistant Enterococci in Denmark by Multiplex PCR and Sandwich Hybridization

Poulsen RL, *et al.* APMIS 1999. 107:404-12.

Gene	Primers(5'->3')	Product(bp)
vanA	GGA AAA CGA CAA TTG CTA TT GTA CAA TGC GGC CGT TA	731
vanB	ACT GGC CTA CAT TCT TAC A AGC GTT TAG TTC TTC CGT	175
vanC1	TCT CCA GAA TAC TCA GTG T ACA TGG CAA CCA ACA TAA G	329
vanC2/3	CCT CAA AAG GGA TCA CTA A TCT TGA TAG GAT AAG CCG A	448
16S rRNA	GGA ATC TTC GGC AAT GGA CAA CCT TGC GGT CGT AC	549

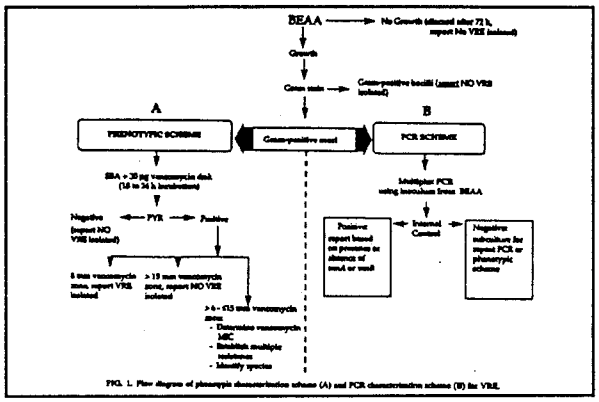
M-PCR with internal control (16S rRNA)



PCR (VII)

Rapid Characterization Schemes for Surveillance Isolates of Vancomycin Resistant Enterococci

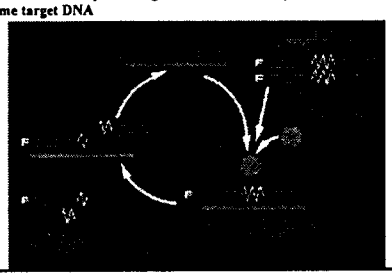
Sahm DF, *et al.* J. Clin. Microbiol. 1997. 35:2026-30.



Cycling Probe Technology

Cycling Probe Technology

- Hybridization of scissle-link probe to target DNA
- Cleavage by RNase H of RNA portion of hybridized probe
- Disassociation of probe fragments
- Accumulation of probe fragments as additional probes are cycled against same target DNA

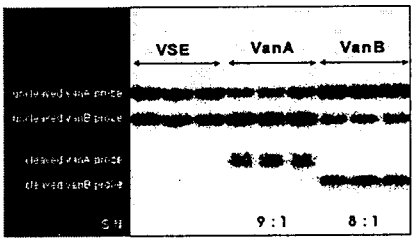


CPT (I)

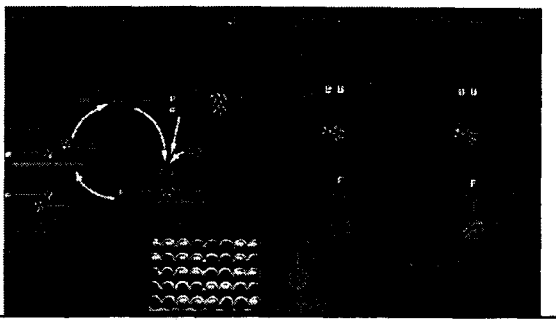
Detection of Vancomycin Resistant Genes *vanA* and *vanB* by Cycling Probe Technology

Modrusan Z, *et al.* Mol. Cell Probes 1999. 13:223-31.

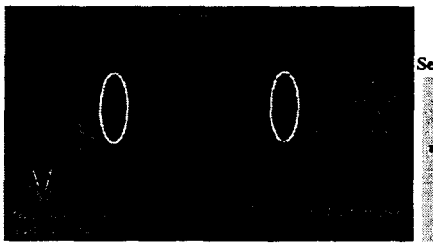
Simultaneous Detection of vanA and vanB with Multiplex CPT



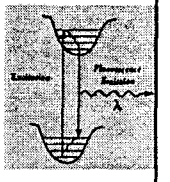
The strategy and results of colorimetric test



A homogeneous CPT assay using fluorescence anisotropy



Several nanoseconds



$$\text{Anisotropy} = \frac{\text{Intensity (vert.)} - \text{Intensity (horiz.)}}{\text{Intensity (vert.)} + 2 \times \text{Intensity (horiz.)}}$$

Advantages of CPT

- Reliable
  - conventional susceptibility tests may misidentify VRE isolates, in particular, VanB with low MIC_{vanco}
- Rapid
  - conventional susceptibility tests require at least 24 hours for results while CPT requires less than 3 hours

探討台灣 VRE 菌株之特性

盧章智

國防醫學院病理學科
三軍總醫院臨床病理科
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Significance of vancomycin-resistant enterococci (VRE) infection (I)

- VRE were first isolated in 1986 from the feces of patients with acute leukemia.
- From 1989 to 1993, the percentage of nosocomial VRE were increased from 0.3% to 7.9% [reported by CDC National Nosocomial Infection Surveillance (NNIS) system] (26-fold increase).
- The percentage of VRE infections in patients in ICU were increased from 0.4% to 13.6% (34-fold increase).

VRE

Significance of vancomycin-resistant enterococci (VRE) infection (II)

- Data from NNIS lists Enterococci as the second most common bacterial cause of nosocomial infection.
- The mortality in cases of VRE infection is 36.6%.
- Can survive on environmental surfaces within hospital for 6-7 days.
- VRE were first isolated in 1996 from the sputum of a patient with pneumonia and bacteremia in Taiwan.

VRE

VRE infection in Taiwan

- ➢VRE were first found in 1995.
- ➢VRE have been isolated from clinical specimens from several medical centers, such as; National Cheng Kung University Hospital, Army Hospital 803, Chang Gung Memorial Hospital, National Taiwan University Hospital , and Tri-Service General Hospital.

	Acquired resistance		Intrinsic resistance
Phenotype	VanA	VanB	VanC
MIC (μg/ml) of			
Vancomycin	64-1000	4-1000	2-32
Teicoplanin	16-512	0.5-1	0.5-1
Expression	Inducible	Inducible	Constitutive (?)
Location of the resistance genes	Plasmids	Chromosome	Chromosome (?)
Mobile elements	Tn*1546*	90-250 kb elements	(?)
Transferability by conjugation	+	-	-
Ligase genes	*vanA*	*vanB*	*vanC-1 vanC-2 vanC-3*
Products	D-Ala-D-Lac	D-Ala-D-Lac	D-Ala-D-Ser
Bacterial species	*E.mesenteroides faecium, E. faecalis, E. avium, E. gallinarum, E. durans, E. mundtii, E. casseliflavus*	*E. faecium, E. faecalis*	*E. gallinarum* (*vanC-1*) *E. casseliflavus* (*vanC-2*) *E. flavescens* (*vanC-3*)

Arthur M, *et al.* J Infect 1996; 32:11.

VRE

Vancomycin-resistant genes (I)

- Two classes of acquired resistance to glycopeptides in enterococci, VanA and VanB, can be distinguished on the basis of resistance or susceptibility to teicoplanin
- Strains with VanB phenotype have various levels of resistance to vancomycin but are susceptible to teicoplanin

VRE

Vancomycin-resistant genes (II)

- VanB strains of *E. faecium* (VREfm) and *E. faecalis* (VREfs) with high levels of resistance to vancomycin (MICs ≥ 64 μg/ml) but sensitive to teicoplanin were found in 1991.
- The resistant gene was transferable and the gene was named *vanB2* because it is similar to *vanB*.

VRE

In 1996, we reported an endemic of VREfm with *van*B2 gene colonizing the intestinal tracts of hospitalized patients in intensive care units

Lu, J. J., C. H. Perng, C. C. Wang, J. H. Lee, and W.H. Lee. An Endemic Vancomycin-Resistant Enterococcus faecium Colonizing the Intestinal Tracts of Hospitalized Patients in Intensive Care Units. Abstract no. CM-17. The 1996 Annual Meeting of the Chinese Association of Microbiology, Taipei, Taiwan, ROC

VRE

All these isolates were found to have the same antibiogram and random amplification polymorphic DNA (RAPD) patterns, suggesting that all 18 isolates were derived from a single clone.

VRE

These findings demonstrate that the multi-drug resistant VREfm with *van*B2 gene can colonize the intestinal tracts of hospitalized patients in ICU and it may be transmissible and that they will emerge as serious nosocomial pathogens.

VRE

Heterogenecity of *vanB2* gene in VRE strains in Taiwan

		VanB138-570		VanBR-BF(463bp)			5'-3' VanB2 (6374bp)				
Hospital	Strain (No)	vanB 433bp	vanB2 1.1kb	PCR	HaeIII	DdeI	DraI	BclI	HaeII	HindIII +NdeI	HindIII +EcoRI
NCKUH	*E.faecium* (9)	-	+	+	2	3	4	4	2	3	4
	E.faecium (1)	-	-	+	2	3	4	4	2	3	4
SLH	*E. faecium* (1)	-	+	+	1	3	4	4	2	ND	4
	E.faecium (1)	-	-	+	ND	ND	4	ND	ND	ND	ND
TSGH	*E.faecium* (5)	-	+	+	2	3	4	4	2	3	4
	E.faecium (7)	-	+	+	2	3	4	ND	ND	3	4
CGMH	*E.faecium* (4)	-	+	+	1	3	4	4	2	3	4
	E. faecalis (2)	-	-	+	ND	3	4	4	2	ND	ND
YT	*E.faecium* (4)	-	+	+	2	3	4	4	2	3	4

VRE

Up to date, we have collected 91 VRE isolates, including 31 of *E. faecalis*, 56 *E. faecium*, 2 *E. gallinarum* and 2 of *E. casseliflavus* from 6 medical centers and 4 district hospitals in Taiwan.

These VRE's were isolated from pus, abscesses, wounds, urine, central venous pressure tips, bile, or blood

Methods

- Antibiotic susceptibility testing by using E-test strips and agar dilution
- Detection of vancomycin-resistant genes by using multiplex PCR
- Typing of VRE by using RAPD & ERIC
- Analysis of *van* genes by autosequencing

VRE

PCR primer sequences

Name	GENE	Sequence (5'-> 3')	Location	Size (bp)	Ref
Van AF	*vanA*	AAT GTG CGA AAA ACC TTG CG	7128-7147	695	Lu
Van AR		CCG TTT CCT GTA TCC GTC C	7786-7804		
Van BF	*vanB*	CAA ATC ACT GGC CTA CAT TC	5363-5382	463	Lu
Van BR		TCT GCA TCC AAG CAC CCG	5808-5825		
Van C1F	*vanC1*	GGT ATC AAG GAA ACC TC	360-376	822	Dutka-Malen
Van C1R		CTT CCG CCA TCA TAG CT	1165-1181		
Van C2F	*vanC2/3*	TAA AGT CAC CTG CGT TGA AG	608-627	312	Lu
Van C2R		ATG CGA GCA AGA CCT TTA AG	900-919		

VRE

Chacaterization of VRE from clinical isolates in Taiwan (I)

Gene	Species	MIC(μg/mL) Van	Tei	No of isolates
vanA	*E. faecium* (n=18)	>256	16-64	11
		>256	8	2
		>256	4	1
		>256	0.75-1.5	4
	E. faecalis (n=29)	>256	16-256	10
		>256	8	6
		>256	4	7
		>256	2	2
		>256	1.0-1.5	3
		96	1	1

VRE

Chacaterization of VRE from clinical isolates in Taiwan (II)

Gene	Species	MIC(μg/mL) Van	Tei	No of isolates
vanB	*E. faecium* (n=38)	>256	0.25-48	28
		128	1.5	2
		64	1-4	2
		48	1	2
		32	1.5	1
		12	1.5	1
		8	1.5	2
		1	1	1
	E. faecalis (n=2)	4	2	1
		3	1.5	1

VRE

Characterization of VRE from clinical isolates in Taiwan (III)

Gene	Species	MIC(μg/mL) Van	Tei	No of isolates
vanC1	*E. gallinarum* (n=2)	>256	96	1
		8	1	1
vanC2	*E. casseliflavus* (n=2)	8	2	1
		4	2	1

VRE

Heterogenecity of *vanB2* gene in VRE strains in Taiwan

Hospital	Strain (No)	VanB138-570		VanBR-BF(463bp)			5'-3' VanB2 (6374bp)				
		vanB 433bp	vanB2 1.1kb	PCR	HaeIII	DdeI	DraI	BclI	HaeII	HindIII +NdeI	HindIII +EcoRI
NCKUH	*E.faecium* (9)	-	+	+	2	3	4	4	2	3	4
	E.faecium (1)	-	-	+	2	3	4	4	2	3	4
SLH	*E. faecium* (1)	-	+	+	1	3	4	4	2	ND	4
	E.faecium (1)	-	-	+	ND	ND	4	ND	ND	ND	ND
TSGH	*E.faecium* (5)	-	+	+	2	3	4	4	2	3	4
	E.faecium (7)	-	+	+	2	3	4	ND	ND	3	4
CGMH	*E.faecium* (4)	-	+	+	1	3	4	4	2	3	4
	E. faecalis (2)	-	-	+	ND	3	4	4	2	ND	ND
YT	*E.faecium* (4)	-	+	+	2	3	4	4	2	3	4

VRE

High prevalence of vancomycin-resistant *Enterococcus faecium* isolates harboring the *vanB2* gene in Taiwan.

Lu, J. J., C. L. Perng, W. M. Chi.

ASM abstract no D/B-226
ASM, annual meeting, 1999

VRE

Summary & conclusions (I)

- Most of VREs (74/91, 81.3%) are highly resistant to vancomycin (MIC?56 痢/ml)
- More than half of *vanA*-bearing VRE isolates (26/47, 55.3%) (either *E. faecium* or *E. faecalis*) had the VanB phenotype (resistant to vancomycin but sensitive to teicoplanin)

Summary & conclusions (II)

- The majority of the VREfm (38/49, 67.9%) isolates were found to contain the *van*B2 gene but not the *van*A gene.
- Most of *van*B-bearing vancomycin-resistant VREfm isolates with high levels of resistance to vancomycin (MICs ≥ 64 μg/ml) but sensitive to teicoplanin (31/38, 81.6%)

VRE

Summary & conclusions (III)

- A fragment of approximately 6370 bp containing the entire putative *van*B2 gene cluster has been sequenced.
- The sequences were found to be homologous to the following genes: *van*YB (94% homology), *van*WB (96% homology), *van*HB (96% homology), *van*B (95% homology), and *van*XB (95% homology).

VRE

Summary & conclusions (III)

- The *van*B-bearing *E. faecium* isolates were found to have the same RAPD and ERIC types, suggesting that they were derived from the same clone.
- Most of *E. faecalis* isolates have different RAPD and ERIC types.

VRE

Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* in Taiwan

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Nosocomial suveillance specimens for VRE

- Routinely collect rectal swabs from patients in ICU from November, 1996.
- Collect VRE isolates from 5 long-term-colonized patients
- Study genotypes and molecular types of isolated VRE strains from long-term-colonized patients

RAPD genotyping

- Two oligonucleotide primers, AP3 (5'-TCA CGA TGC A-3') and ERIC1R (5'-ATG TAA GCT CCT GGG GAT TCA C-3'), were selected for arbitrarily for experiments

General data of vancomycin-resistant enterococcal genotypes and molecular types in long-term-colonized patients (I)

Patient (no)	Collection date	Specimen ID	Genotypes	Molecular types
A (24)	86.01.05	M823	B	IA
	86.01.12	M826	B	IA
	86.01.27	M831	B	IA
	86.02.02	M832	B	IA
	86.02.10	M835	B	IA
	86.02.21	M842	B	IA
	86.03.02	M844	B	IA
	86.03.08	M848	B	IA
	86.03.15	M901	B	IB
	86.03.22	M903	B	IIB
	86.03.28	M906	B	IA
	86.03.29	M908#	B	IA
	86.04.05	M909#	B	IIIC
	86.04.04	M910	B	IIIC
	86.04.14	M914	B	IA
	86.04.20	M915	B	IA
	86.04.28	M916	B	IA
	86.05.06	M919	B	IA
	86.05.05	M921	B	IA
	86.05.22	M930	B	IA
	86.05.22	M931*	B+C1	IVD
	86.05.29	M933	B	IB

General data of vancomycin-resistant enterococcal genotypes and molecular types in long-term-colonized patients (II)

Patient (no)	Collection date	Specimen ID	Genotypes	Molecular types
B (7)	85.10.13	M715	B	IA
	85.11.02	M720	B	IA
	85.11.09	M727	B	IA
	85.11.16	M735	B	IA
	85.11.29	M743	B	IA
	85.11.29	M744	B	IA
	85.12.10	M804	B	IA
	85.12.17	M816	B	IA

General data of vancomycin-resistant enterococcal genotypes and molecular types in long-term-colonized patients (III)

Patient (no)	Collection date	Specimen ID	Genotypes	Molecular types
C (10)	86.06.02	M942	B	IA
	86.06.10	M944	B	IA
	86.06.23	L705	B	IA
	86.06.29	L706	B	IIID
	86.07.02	L708	B	IA
	86.07.16	L715	B	IA
	86.07.23	L730	B	IA
	86.08.07	L747	B	IA
	86.07.30	L739	B	IIID
	86.08.14	L812	B	IA

General data of vancomycin-resistant enterococcal genotypes and molecular types in long-term-colonized patients (IV)

Patient (no)	Collection date	Specimen ID	Genotypes	Molecular types
D (8)	85.10.13	M714	B	IA
	85.11.02	M719	B	IA
	85.11.09	M729	B	IA
	85.11.16	M733	B	IA
	85.11.25	M737	B	IA
	85.12.01	M746	B	IA
	85.12.16	M809	B	IA
	86.01.11	M828	B	IA

General data of vancomycin-resistant enterococcal genotypes and molecular types in long-term-colonized patients (V)

Patient (no)	Collection date	Specimen ID	Genotypes	Molecular types
E (15)	85.11.25	M740	B	IA
	85.12.02	M748	B	IA
	85.12.09	M749	B	ID
	85.12.09	M803	B	IIID
	85.12.22	M810	B	IA
	85.12.30	M818	B	IA
	86.01.01	M824	B	IA
	86.01.13	M827	B	IA
	86.01.20	M829	B	IA
	86.01.27	M830	B	IA
	86.02.03	M833	B	IA
	86.02.10	M836	B	IA
	86.02.16	M838	B	IA
	86.02.23	M840	B	IA
	86.03.04	M845	B	IA

Vancomycin-resistant *Enterococcus faecium* strains in Taiwan

- Collect 50 vancomycin-resistant *E. faecium* isolates (VREfm) from 6 hospitals in Taiwan
- Perform antibiotic susceptibilty testing, genotypes, and molecular types of isolated VREfm strains

Hospital	strains	*vanB* (No.)	Type IA (No.)
A	23	12	9
B	2	2	2
C	1	0	0
D	9	5	5
E	2	2	2
F	13	13	9

Characteristics of glycopeptide-resistant *Enterococcus faecium* isolated in Taiwan (I)

Hospital (No.)	Specimen ID	Source	MIC(μg/mL) Tei	MIC(μg/mL) Van	Genotypes *vanA*	Genotypes *vanB*	Molecular patterns
A (23)	F901	stool	0.25	>256	-	+	IA
	F909	blood	0.5	>256	-	+	IA
	F910	blood	0.5	>256	-	+	IA
	F914	wound	32	>256	+	-	IA*
	F916	wound	32	>256	+	-	IA*
	F918	stool	16	>256	+	-	II
	F924	urine	32	>256	+	-	IA*
	F934	urine	64	>256	+	-	IA*
	F935	urine	64	>256	+	-	IA*
	F937	urine	32	>256	+	-	IA*
	I716	cvp tip	0.5	>256	-	+	IA
	I741	blood	1.5	>256	-	+	ID
	I742	urine	2	>256	-	+	ID
	I746	urine	1	>256	-	+	IA
	P6-9	urine	48	>256	+	-	IA*
	P6-25	urine	32	>256	+	-	IA*
	P6-31	urine	0.75	24	-	+	IA
	P6-41	urine	32	>256	+	-	IA*
	P7-16	blood	48	>256	+	-	IA*
	M909	wound	1.0	>256	-	+	IVE
	M941	blood	1.0	>256	-	+	IA
	TSGH1	tip	1.0	>256	-	+	IA
	TSGH2	wound	1.0	>256	-	+	IA

Characteristics of glycopeptide-resistant *Enterococcus faecium* isolated in Taiwan (II)

Hospital (No.)	Specimen ID	Source	MIC(μg/mL) Tei	MIC(μg/mL) Van	Genotypes *vanA*	Genotypes *vanB*	Molecular types
B (2)	Y1	urine	1.5	>256	-	+	IA
	2-50	wound	1	>256	-	+	IA
C (1)	1C	urine	4	>256	+	-	VD
D (9)	VRE-1	wound	1	4	-	+	IA
	VRE-2	wound	0.5	>256	+	-	IB
	VRE-3	wound	1	8	-	+	IA
	VRE-4	wound	1	>256	+	-	IB
	VRE-7	wound	1	>256	+	-	IB
	VRE-8	wound	1	32	-	+	IA
	24-24	pus	1.5	>256	-	+	IA
	2206	wound	0.5	256	+	-	IB
	4248	wound	1	64	-	+	IA

Characteristics of glycopeptide-resistant *Enterococcus faecium* isolated in Taiwan (III)

Hospital (No.)	Specimen ID	Source	MIC(μg/mL) Tei	MIC(μg/mL) Van	Genotypes *vanA*	Genotypes *vanB*	Molecular types
E (2)	475	urine	1	>256	-	+	IA
	476	wound	1	3	-	+	IA
F (13)	CKU-2	pus	0.5	48	-	+	IA
	CKU-4	pus	0.5	>256	-	+	IA
	CKU-5	pus	0.5	>256	-	+	IA
	CKU-6	pus	1	>256	-	+	IIE
	CKU-8	wound	1	128	-	+	IA
	CKU-10	pus	1.5	>256	-	+	IA
	CKU-11	urine	2	>256	-	+	IA
	CKU-12	pus	1	>256	-	+	IA
	CKU-13	pus	1	>256	-	+	IA
	CKU-14	stool	4	>256	-	+	IIC
	CKU-16	pus	4	>256	-	+	IIC
	CKU-20	urine	0.5	>256	-	+	IA
	CKU-21	urine	4	>256	-	+	IIIE

Summary and conclusions (I)

- The results demonstrated that long-term colonized VREfm had highly resistant to vancomycin (MIC > 256 mg/L).
- They all had *vanB* genotype but 2 of them contained *vanC1* gene in addition to *vanB* gene.
- The results also showed that majority of VREfm (88%, 44/50) from clinical isolates also had high-level resistance to vancomycin (MIC > 64 mg/L), and majority of these VREfm (64%, 32/50) had *vanB* gene.

Summary and conclusions (II)

- The IA type is the most common type of VREfm in long-term colonized patients and in clinical isolates.
- We found that the VREfm isolated from long-term colonized patients are stable and can transmit the van gene to other enterococci in intestinal tract of the patients.

Summary and conclusions (III)

- The results suggest that the IA type *E. faecium* carrying *vanB* gene might have already been transmissible to everywhere in Taiwan.
- The source of this strain remains to be determined.

VRE 院內感染籌劃

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Prevention and control of vancomycin-resistant enterococci (VRE)

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Chih-Chien Wang

1. Enterococcus

(1). Formerly classified in the genus Streptococcus. ***E. facalis*** **and** ***E. faecium*** are of medical importance.
(2). Gram-positive cocci, cells often in pairs and chains; more ovate appearance; capable of aerobic and anaerobic respiration.
(3). On blood agar may produce α,β, or no hemolysis. Bile tolerant; relative heat tolerant and salt tolerant. Hydrolyse aesculin and arginine.
(4). **UTI, endocarditis, sepsis** after surgery and in the immunocompromised.
(5). Normal habitat is the **gut of humans** and animals. Most infections thought to be **endogenously** acquired but **cross-infection** may occur in hospital.
(6). No toxins but some virulence factors such as adhesins demonstrated.
(7). Penicillin used in combination with aminoglycosides for synergy in sever infection, but now, resistance to aminoglycoside and penicillin increasing, vancomycin is indicated.
(8). **Vancomycin-resistant enterococci (VRE)** is a medical problems in recent years.

2. Clinical importance of Enterococci

(1). Data from NNIS lists Enterococci as the **second** most common bacterial cause of nosocomial infection.
(2). NNIS data showed that, from 1989 (0.3%), through 1993, nosocomial VRE increased **20-fold** to 7.9%.
(3). Can survive on **environmental surfaces** within hospital **for 6-7 days**.

3. Risk factors for the acquisition of VRE

(1). **Abdominal surgical procedures.**
(2). **Multiple antibiotic therapy.**
(3). **Preexposure to vancomycin.**
(4). **Prolonged hospital stay**

4. Types of glycopeptide resistance in Enterococci

	VanA	VanB	VanC
Vancomycin MIC (μg/mL)	≥64	≥4	2–32
Teicoplanin MIC (μg/mL)	≥16	0.5–1	0.5–1
Transferable	Yes	Yes	No
Inducible	Yes	Yes	No
Species	*E. faecium* *E. faecalis* *E. avium* *E. casseliflavus* *E. durans* *E. gallinarum*	*E. faecium* *E. faecalis*	*E. gallinarum* *E. casseliflavus*

MIC, minimum inhibitory concentration.

5. Detection of VRE

Willey BM evaluated many methods to detection of 155 *Enterococcus* spp. Isolates with varying degree of vancomycin resistance. (J Clin Microbiol 1992;30:1621)

(1). Disk diffusion test: only 5.8% minor errors.
(2). The Vitek system:98% specific and 73% sensitive.
(3). The Microscan system: 98% specific, 93% sensitive.
(4). Agar dilution studies: 100% sensitive and specific.

6. Prevention and control of VRE

(1). Isolation in a single room or cohort in the same room.
(2). Wear gloves when entering the room.
(3). Wear a gown when contact patient or environmental surfaces.
(4). Remove gloves and gown before exiting the room.
(5). Wash hand immediately with antiseptic solution.
(6). Clean and disinfect contaminated, reusable items, such as stethoscope.
(7). All roommates must sent a rectal swab/or stool for VRE culture.
(8). Three negative culture, at least one week apart, before a patient removed from isolation precautions.
(9). Rapid identification of patients have VRE.
(10). Flag the chart or computer records.

7. The guidelines in vancomycin use

(1). Treatment of serious infections caused by β-lactam-resistant gram-positive organisms.
(2). Treatment of infectious caused by gram-positive microorganisms in patients who have serious allergies to β-lactam antimicrobials.
(3). When antibiotic-associated colitis fails to respond to metronidazole therapy or is severe and potentially life-threatening.
(4). Prophylaxis, as recommended by the American Heart Association, for endocarditis following certain procedures in patients at high risk for endocarditis.
(5). Prophylaxis for major surgical procedures involving implantation of prosthetic materials or devices at institutions that have high rate of infections caused by MRSA or MRSE.

8. *Staphylococcus aureus* with reduced susceptibility to vancomycin

(1). **In 1996**, the first documented case of infection caused by a strain of ***S. aureus*** with intermediate levels of resistance to vancomycin (**VISA, MIC= 8μg/ml**) was reported from **Japan**.
(2). **In July 1997**, VISA-associated peritonitis was diagnosed in a patient who was being treated with long-term ambulatory peritoneal dialysis in the **United States**. The patient had been treated with multiple courses of both intraperitoneal and intravenous vancomycin for repeated MRSA peritonitis.
(3). Epidemiological and laboratory investigations are under way to assess the risk for **person-to-person transmission** of VISA and to determine the **mechanism(s)** by which these strains develop resistance.

三軍總醫院加護中心VRE感染及移生病患加強監視及管制措施

八十五年十月院內感染管制委員會制訂

1.當細菌室分離出VRE（Vancomycin-Resistant-Enterococci）菌株，需立即通知病房及院內感染管制小組，病房需採取管制措施，並在病歷首頁標示及病人單位掛上“VRE”牌子以提醒工作人員注意。

2.對所有證實有VRE感染或移生之病患均應採取隔離措施，病患需住進單人房間或集中在同一房間。

3.進入病室若需接觸病患或環境中任何物品時，均需戴手套並穿隔離衣。

4.病患受感染或移生部位之分泌物、痰液有可能飛濺起來時應戴口罩。

5.離開病室需先脫隔離衣及手套，然後立即以消毒劑洗手。

6.VRE病患要有專用的聽診器，溫度計及血壓計，當病人出院或死亡這些醫療用品需做清潔及消毒。

7.照顧VRE病患的醫護人員，盡量不要再安排去照顧非VRE的病患。

8.ICU環境消毒：

(1)地面：每天應以稀釋100倍之漂白水拖地。

(2)傢俱、床舖、儀器、電腦、電話、桌面、門把、病歷夾等平常每天用清潔劑擦拭，每週應以稀釋10倍漂白水擦拭一次。

(3)空調：濾網（心）應每半年更換，出口處應每個月清潔之。

9.移生病患監視（目前僅針對W31、W32實施）

病患入加護中心之第一天及每週，均採糞便或肛門拭子培養，以期早日偵測，並做VRE之管制措施，當連續3次培養均為陰性才解除隔離措施，但仍繼續監測直至離開加護中心為止。

10.感染病患監視：

發燒病患立即送血液、尿液、痰液、傷口及導管尖端培養，儘快找出感染源，以便使用適當之藥物治療。

11.各加護中心護理長及副護理長應密切注意本監視及管制措施，並在每次院內感染管制委員會提報。