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行政院衛生署疾病管制局九十五年度科技研究發展計畫

台灣醫院院內感染細菌之基因型及分子流行病學

Genotyping and molecular epidemiology of bacterial pathogens causing hospital acquired infections in Taiwan hospitals

研 究 報 告

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目　　錄

一、 摘要

（一）中文摘要

細菌抗藥性是一全球重視之公衛問題，而醫院之不斷使用抗生素，則導致細菌在選擇性壓力下產生抗藥性菌株，造成院內感染細菌大多具多重抗藥性，故引起院內感染細菌的抗藥性最嚴重。此計畫使用國家衛生研究院臨床研究組之全國微生物抗藥性監測計畫(Taiwan Surveillance of Antimicrobial Resistance, TSAR)菌種，進行抗藥基因序列及分子流行病學之研究，研究方法包含：脈衝電泳法(Pulsed-Field Gel Electrophoresis, PFGE)、多位基因序列分析法(Multi Locus Sequence Typing, MLST)來研究其分子流行病學之演變。並使用聚合酶連鎖反應(polymerase chain reaction, PCR)及去氧核醣核酸序列分析法(DNA sequencing)來研究其抗藥機制及毒性因子。

此計畫今年最主要之研究對象為抗甲氧苯青黴素金黃色葡萄球菌(MRSA)，調查之菌株為 TSAR I ～IV 四期間之院內感染 MRSA 共 226 多株，包含 1998 年(TSAR I)之 95 株、2000 年(TSAR II)之 39 株、2002 年(TSAR III)之 38 株及 2004 年(TSAR IV)之 54 株。結果顯示，國內引起院內感染之 MRSA，可分為四個主要群落，最常見的群落為 pulsotype A 的菌，佔了 226 株菌中 174 株(77.0%)。Pulsotype A 的菌抗藥性最高，幾乎 100%對 ciprofloxacin、clindamycin、erythromycin、gentamicin、tetracycline 及 trimethoprim/sulfamethoxazole (SXT)皆具抗性。另一群基因型 pulsotype C MRSA 群落，共佔了 5.6% (9 株)。Pulsotype C 群落值得注意的是其所具的 PVL 毒性因子，PVL 是破壞白血球之毒素，為最近幾年在不同國家引起社區感染及群突發案例之 MRSA 的一主要特徵，主要引起皮膚感染及致命嚴重肺炎。此菌群在國內院內感染病人找到，表示其已經侵入醫院，可能造成嚴重疾病。另一個 pulsotype D 亦有增加的驅勢(從 1998 年之 0%，於 2004 年增加至 20%)。這些結果顯示國內之 MRSA 流行病學正在演變，應進一步調查其在各醫院所引成之疾病及病人特性，以便找出其致病之危險因子，及協助制定疾病治療及感控措施之方針。

關鍵詞：院內感染、抗藥細菌、分子流行病學、抗甲氧苯青黴素金黃色葡萄球菌(MRSA)

（二）英文摘要

Background and Purpose

Antimicrobial resistance is an increasing public health problem worldwide. Organisms causing hospital acquired infections have the highest rates of antimicrobial resistance. This project utilized isolates collected in the 4 rounds of Taiwan Surveillance of Antimicrobial Resistance (TSAR) project in 1998, 2000, 2002, and 2004 to study the molecular epidemiology and mechanisms of resistance in methicillin resistant *Staphylococcus aureus* (MRSA) causing hospital acquired infections in Taiwan.

Methods

Pulsed-Field Gel Electrophoresis, multilocus sequence typing, and DNA sequencing were used to study the evolution and relatedness of the strains. Polymerase chain reactions were used to study the genes (SCC*mec*) responsible for methicillin resistance and to detect the presence of PVL toxin genes.

Results

MRSA comprised 68 to 80% of the *S. aureus* causing hospital acquired infections (HAI) in Taiwan. A total of 226 MRSA isolates causing HAI from the 4 study years were studied for their molecular characteristics and resistance mechanisms. Four main pulsed field gel electrophoresis types (pulsotypes) were found. The majority (77%, 174 isolates) of the isolates belong to pulsotype A, and there were 19 isolates (8.4%) in pulsotype B, 9 isolates (4.0%) in pulsotype C, and 15 isolates (6.6%) in pulsotype D. Although pulsotype A isolates remained the predominant clone in all 4 years, accounting for 90.5% of the MRSA in 1998, only 57.4% of 2004 MRSA isolates belong to pulsotype A. In contrast, pulsotype D was not found in 1998 but increased to account for 20.4% of the MRSA isolates causing HAI in 2004. The proportions of pulsotypes B and C also changed over the years. Isolates in pulsotypes B and C share characteristics usually found in community-onset MRSA (C-MRSA), such as the possession of SCC*mec* types IV (pulsotype B & C) and PVL toxin (in pulsotype C).

Conclusions

These results indicated that the epidemiology of MRSA causing hospital infections is changing in Taiwan and C-MRSA may be migrating into the hospital environment. Further studies on disease spectrum and risk factors associated with each clone.

Key Words: Hospital acquired infection, antimicrobial resistance, methicillin resistant *Staphylococcus aureus* (MRSA), molecular epidemiology

本文

(一) 前言 (Background)

細菌抗藥性是一全球重視之公衛問題[1,2]。病人可因感染抗藥細菌而治療無效或需使用較昂貴之後線藥、及住院日之加長，而增加許多醫療費用和病患死亡率[3,4]，不只加深病人及其家屬之經濟及心理負擔與後遺症，也造成國家社會之經濟與公衛型態之負面影響。進年來，因醫療制度之改善，病人住院時之各種延續生命及維持器官機能的侵犯性醫療器材之使用，如：呼吸管、導管等，及抗生素之大量與長期使用，都造成病人住院時被抗藥細菌感染之機會。而醫院之不斷使用抗生素，則導致細菌在選擇性壓力(selective pressure)下產生抗藥性菌株，這些抗藥菌長期在醫院之選擇性環境中維持及繁殖，且可一再突變或從其他細菌及不同菌種獲得抗藥性基因，造成院內感染細菌大多具多重抗藥性。

國家衛生研究院臨床研究組於 1998 年即開始進行「全國微生物抗藥性監測計畫(Taiwan Surveillance of Antimicrobial Resistance, TSAR)」，監測對象為台灣北中南東地區之醫學中心及區域醫院由加護病房、普通病房及門診病人所分離出的病原細菌[5-7]，至今已完成四期。TSAR 及國內研究資料顯示，台灣細菌之嚴重抗藥性不只是在醫院內，亦在于門診病人中。但比較由不同醫院部門分離出的細菌之抗藥性，則以引起院內感染細菌的抗藥性最嚴重，舉例來說， TSAR IV 中抗甲氧苯青黴素金黃色葡萄球菌(Methicillin Resistant *Staphylococcus aureus*; 以下簡稱 MRSA)佔金黃色葡萄球菌的比率是 56%，其中由門急診病人分離出之金黃色葡萄球菌中，MRSA 的比率為 40%，但引起院內感染之金黃色葡萄球菌中，MRSA 的比率則高過 75%[8]。

抗藥菌之抗藥機制，包含抗生素目標之突變使抗生素與目標之親合力減低，亦可經由抗藥基因轉錄之胺素可破解抗生素而造成抗生素無效。抗藥基因可位於細菌的染色體及質體(plasmid)上，而同一個質體上經常具有多重抗藥基因。另有些抗藥基因可存在轉

位子(transposon)上，轉位子可在質體與染色體間互相跳躍來傳遞抗藥基因，另外一名為嵌入子(integron)的基因，則可讓外來的抗藥性基因嵌入質體或染色體內，這些基因是導致多重抗藥性菌之主要原因，而引起院內感染突發之主要細菌亦為多重抗藥菌[9-13]。

TSAR 及國內研究資料顯示，國內臨床最常見之院內感染細菌為：大腸桿菌(*Escherichia coli*)、克雷白氏肺炎桿菌(*Klebsiella pneumoniae*)、綠膿桿菌(*Pseudomonas aeruginosa*)、鮑氏不動桿菌 (*Acinetobacter baumannii*)、金黃色葡萄球菌(*Staphylococcus aureus*)、及腸球菌(*Enterococcus* spp.)。這些菌中，又以具廣譜酶(extended-spectrum β -lactamase; ESBL)的大腸桿菌(ESBL producing *E. coli*)、具廣譜酶的克雷白氏肺炎桿菌(ESBL producing *K. pneumoniae*)、對 imipenem 具抗性及全抗性的綠膿桿菌(Imipenem resistant and pan-drug resistant *Pseudomonas aeruginosa*)、對 imipenem 具抗性的鮑氏不動桿菌(imipenem resistant *A. baumannii*)、對甲氧苯青黴素具抗性的金黃色葡萄球菌(Methicillin resistant *Staphylococcus aureus;* MRSA)、及對萬古黴素具抗性之腸球菌(Vancomycin resistant *E. faecalis* 及 *E. faecium*)之抗藥菌更嚴重[14,15]。

了解抗藥菌之抗藥機制及其流行病學，是探討抗藥菌傳播途徑及其抗藥性維持或增加原因之方法之一，可協助防止抗藥菌之進一步擴散及衍生。比較不同年度所收集之菌種之抗藥機制及分子流行病學，亦可增加對抗藥菌演變之了解，以採取適當防禦措施 此研究計畫將建立抗藥基因庫及抗藥菌分子流行病學系統。此研究所使用之 TSAR 菌種及基因序列亦將逐年分批一份給疾病管制局做為研究資源之備份及基因序列資料庫之建立，以做為與將來抗藥細菌感染突發調查及研究、以探討及比較其演變及防治其擴散方策之依據。

(二) 材料與方法 (Materials and Methods)

1. 研究對象：

此計畫使用由 1998 年至 2004 年四期[全國微生物抗藥性監測計劃(Taiwan Surveillance of Antimicrobial Resistance, TSAR)]收集到的各醫院從病人檢體分離出之院內感染致病細菌為研究對象，包含大腸桿菌(*Escherichia coli*)、克雷白氏肺炎桿菌(*Klebsiella pneumoniae*)、綠膿桿菌(*Pseudomonas aeruginosa*)、鮑氏不動桿菌(*Acinetobacter baumannii*)、金黃色葡萄球菌(*Staphylococcus aureus*)、及腸球菌(*Enterococcus* spp.)。此計畫今年研究對象為引起院內感染之抗甲氧苯青黴素金黃色葡萄球菌 (Methicillin resistant *S. aureus,* MRSA)，具廣譜酶之大腸桿菌及克雷白氏肺炎桿菌[Extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumonia*]，及對抗萬古黴素之腸球菌(Vancomycin resistant enterococci, VRE)。

2. 研究及分析方法：

A). 使用世界衛生組織之 Whonet 分析軟體[22]，分析這些細菌對多種抗生素之抗藥性，並使用 Epi Info 6.04 軟體(CDC, Atlanta, GA)統計學比較其不同年度抗藥趨勢。

B). 依美國國家實驗室規範(CLSI/NCCLS)的標準，測出院內感染的大腸桿菌及克雷白氏肺炎桿菌中那些是具廣譜酶之菌(ESBL confirmatory testing)[23]。首先挑選大腸桿菌及克雷白氏肺炎桿菌中，其 ceftazidime, cefotaxime, 或 aztreonam 抗生素的最低抑菌濃度 (Minimum inhibitory concentration, MIC) 大於或等於 2 μg/ml，即懷疑是 ESBL 之菌。測試方法步驟如下：從培養 18-24 小時的培養皿挑取 3-5 個相同菌落至 5 ml 食鹽水，調整菌液濃度至 0.5 McFarland 標準，將菌落畫至 Mueller-Hinter 後，同時測定 ceftazidime，cefotaxime 及這兩種藥物加入 clavulanic acid 的最低抑菌濃度。如加入 clavulanic acid 的 ceftazidime 或 cefotaxime 之比沒加入 clavulanic acid 之 ceftazidime 或 cefotaxime 之 MIC 至少降低八倍，即確認是 ESBL producing 之菌。

C). 抗藥基因及分子流行病學研究方法包含：脈衝電泳法(Pulsed-Field Gel Electrophoresis, PFGE)來探討表一中所有抗藥性細菌之去氧核醣核酸基因型(pulsotype)的相關性[24,25]。用多位基因序列分析法(Multi Locus Sequence Typing, MLST)來定序 MRSA 的 7 個內部基因，找出此株菌相對應的多位基因序列分型(Sequence type, ST)，以便追蹤國內 MRSA 之演變[26]。並由脈衝電泳法(PFGE)及多位基因序列分析法(MLST)結果，挑選主要脈衝電泳型別(pulsotype)及序列型別(Sequence type, ST)菌類

iv. 以聚合酶連鎖反應(polymerase chain reaction, PCR)及去氧核醣核酸序列分析法(DNA sequencing)來研究其抗藥機制，包含 MRSA 之 SCC*mec* typing 及 Panton-Valentine leukocidin (PVL) toxin gene detection，VRE 之 vanA 與 vanB 之測定。

實驗方法簡述如下：

i. 脈場膠電泳法分型(Pulsed field gel electrophoresis, PFGE)

此實驗根據美國 CDC 建立之 MRSA PFGE 標準操作[ref]，從隔夜培養之羊血平板上挑取單一菌落，以 1 ml 溶液調整菌液濃度，取等體積之 1.6%低熔點的洋菜膠（agarose）與菌液均勻混合，分裝入填充模型（plug mold），凝固後取出填充物（plug）將之分解，置入 buffer，50℃隔夜振盪，洋菜膠填充物（plug）以 buffer 清洗；再移到含 TE 溶液之試管，切下 1.0 到 1.5 mm 厚的薄片，置入含 250 ul 之限制腖溶液內含 20 單位之限制酵素 *Sma*I 之反應溶液；經 DNA 分解 agarose plugs 放入 TE buffer 37° 1 小時，plug 插入 1% agarose gels，在 0.5x TBE buffer 將切斷的片段以電泳槽 CHEF-Mapper 跑膠質，不同菌使用之菌液濃度、buffer、限制腖、電泳變換時間、及電泳時間不同，其分子量指標亦不同；電泳後以 0.5 μg/mL ethidium bromide 染色 30 分鐘，清洗後以紫外光照射顯像。用 BioNumerics 的 PFGE 分析專用軟體進行分型圖譜 dendrogram 電泳相似性（similarities）比對，當這些菌株之 similarity ≧80%時，即被認為來自相關菌源，稱為 pulsotype (A to D)，而各 pulsotype 中如有少數 DNA banding 差別，則稱為 subtype (如：A1, A2 或 C1, C2 等)。

ii. 聚合酶連鎖反應(polymerase chain reaction, PCR)

過夜培養之培養基(非選擇性培養基)上挖取約 $2x10^9$ cell 的細菌萃取 DNA，於 0.2 ml 的 PCR 專用薄壁離心管內加入(每一個檢體) ddH_2O、1X buffer、10 picomole 核酸引子對(primers)(不同抗藥基因各有其目標之 primer 序列)、2.5 nM dNTP、0.2 U Taq polymerase、細菌 DNA，放入 Thermocycler，經過 30 至 35 cycles 的 denaturing, annealing, elongation，放 5 ul 之 PCR 反應溶液與 loading dye 於洋菜膠，電泳後，用 EtBr 染色照相判斷結果。確認約 DNA 產物後，純化剩餘之 PCR 產物以進行核酸定序步驟。Staphylococcal cassette chromosome *mec* (SCC*mec*) typing 之方法參考 Ma et al., [27]. VRE 之 vanA 與 vanB 之 PCR 測定方法參考 Dulka et al., [28]

iii. 多位基因序列分析法(MLST)

抽取測試之金黃色葡萄球菌的 DNA，每株菌分別進行下列 7 組引子(primer)之聚合酶酵素鏈鎖反應(PCR)：*arcC, aroE, glpF ,gmk, pta, tpi, yqiL*，PCR 反應溶液純化配後，序列反應後，用組合序列之軟體先將 forward 及 reverse 端組合(如 Chromas、VectorNTI 等)之後，進行核酸序列比對，將每株菌所得的 7 段序列貼上 MLST 網站(http://saureus.mlst.net/sql/multiplelocus.asp)，以找出此株菌相對應的多位基因序列分型(Sequence type, ST)[26]。

(三、四) Results and Discussion (結果　與　討論)

- **Infection sites of nosocomial bacteria.**

Table 1A lists the specimen source of 7 major bacterial species causing hospital acquired infections in Taiwan in TSAR I (1998), TSAR II (2000), TSAR III (2002), and TSAR IV (2004). Among these, the most common specimen source for *E. coli* was urine (54.8%, 142/259), followed by blood (21.2%, 55/259). In *K. pneumoniae*, the most common specimen sources were blood (31.6%, 60/190), urine (27.9%, 53/190), and respiratory tract specimens (23.2%, 44/190). In *A. baumannii*, respiratory specimens accounted for nearly half (45.6%, 94/206) of the specimens, followed by blood (23.3%, 48/206); while in *P. aeruginosa*, the most common specimen source was respiratory (36.9%, 109/295) followed by urine (26.1%, 77/295). For *S. aureus*, the most common specimens were blood (38.9%, 118/303), respiratory (25.4%, 77/303), and wound (21.8%, 66/303). Urinary tract was the most common infection site for *Enterococcus* species, accounting for 39.2% (40/102) of *E. faecalis* and 54.5% (18/33) of *E. faecium* in HAI caused by these two species. Thus the most common hospital acquired infections caused by these bacteria included urinary tract and respiratory tract infections. Bacteremia was also common. In addition, *S. aureus* also commonly caused wound infections also in hospitalized patients.

- **Infection sites of bacteria isolated from patients after 3 days of hospitalization but were not considered as nosocomial infections.**

The specimen sources of the same 7 common bacterial species isolated from patients after 3 days hospitalization but were not considered as HAI are listed in Table 1B. Analysis was not done on TSAR I isolates because of lack of admission dates. One noteworthy difference in the specimen sources of these isolates from HAI isolates is the

increased proportion of respiratory specimens especially in *K. pneumoniae, A. baumannii,* and *P. aeruginosa*. For example, respiratory tract specimens accounted for 44.4% (127/286) of *K. pneumoniae* isolates in this patient group compared to 23.2% (44/190) in HAI patients. Wound specimens also accounted for larger proportions in most of these isolates, especially in *S. aureus* (38.6%, 189/490 vs. 21.8%, 66/303 in HAI isolates). Conversely, blood isolates comprised smaller proportions in 6 of these species (except *E. coli*) in this category. These results indicated that some of these isolates may be from patients colonized by these species after being hospitalized for a while. Microbiology laboratory can play an important role in assisting and communicating with physicians in determining the importance of these culture results to avoid overuse of antimicrobials.

- **Prevalence of antimicrobial resistance in nosocomial bacteria in different years.**

The prevalence of antimicrobial resistance among the 7 common bacterial pathogens causing hospital acquired infections (HAI) is listed in Tables 2A and 3A. Comparison of their resistance trends over time was also made. There were significant increases in resistance of *E. coli* to several β-lactam antimicrobials and fluoroquinolone ciprofloxacin . The same trend was observed in *K. pneumoniae*. These increasing resistance may be associated with the increase of extended-spectrum β-lactamase (ESBL) producing strains in these two species. No significant change in resistance was observed in *P. aeruginosa*. There were even significant decrease in resistance to aminoglycosides amikacin and gentamicin in *P. aeruginosa*. The most noteworthy increase in resistance in *A. baumannii* was to carbapenem imipenem (from <2% before 2002 to 12% in 2004). Another noteworthy change is in resistance of *S. aureus* and MRSA to tetracycline and SXT, which showed significant decreasing trend.

- **Prevalence of antimicrobial resistance in bacterial species recovered from patients after 3 days hospitalization (excluding HAI isolates).**

The prevalence of antimicrobial resistance among these 7 common bacterial pathogens isolated from patients hospitalized for 3 days or longer but were not considered as HAI is listed in Tables 2B and 3B. Although these isolates in general have lowered rates of resistance to some antimicrobials than HAI isolates, their resistance prevalence is still very high. One noteworthy difference was in *A. baumannii* from these patients, the resistance of which was even higher than those from HAI, likely due to the higher imipenem resistance (18.2% vs. 12.2% in HAI isolates) in this group of isolates since most imipenem resistant *A. baumannii* are also multiply resistant to other antimicrobials. Resistance of *S. aureus* and MRSA to tetracycline and SXT in this group of isolates also showed significant decreasing trend, same as the HAI isolates.

- **Epidemiology of Methicillin resistant *S. aureus* (MRSA) in Taiwan.**

The main species studied for the project this year is MRSA, which comprised 68 to 80% of the *S. aureus* causing HAI (Table 3). Of noteworthy is that although there was no significant change in the rate of methicillin (oxacillin) resistance in *S. aureus* from the 4 study periods, there were significant decreases in resistance to ciprofloxacin, tetracycline, and trimethoprim/sulfamethoxazole (SXT). When the susceptibility patterns were compared among MRSA isolated in different years from the 4 rounds of TSAR, significant decreases in resistance to ciprofloxacin, tetracycline, and SXT were also found. All MRSA isolates were resistant to erythromycin.

We next performed molecular typing on MRSA isolates causing HAI from the 4 study years. A total of 226 MRSA isolates were studied, including 95, 39, 38, and 54 isolates from 1998, 2000, 2002, and 2004, respectively. Four main pulsed field gel electrophoresis types (pulsotypes) were found among these 226 isolates. The majority (77%, 174 isolates) of the isolates belong to pulsotype A (Fig. 1). There were a total of 19 isolates (8.4%) in pulsotype B, 9 isolates (4.0%) in pulsotype C, and 15 isolates (6.6%) in pulsotype D. The

remaining 9 isolates were different from pulsotypes A to D and most were distinct from each other.

Differences in the characteristics of these 4 main pulsotypes are listed in Table 4. The resistance patterns, SCC*mec* types, sequence type (ST) from MLST, and the presence of Panton-Valentine leukocidin (PVL) gene were compared. There were significant differences in the resistance profiles in isolates from these 4 main pulsotypes. The most common pulsotype (A) had the highest rates of resistance. Almost all 174 pulsotype A isolates were resistant to ciprofloxacin (CIP, 100%), clindamycin (CLI, 93.1%), erythromycin (ERY, 100%), gentamicin (GEN, 99.4%), tetracycline (100%), and SXT (97.1%). In contrast, although all 19 pulsotype B isolates were also CLI and ERY-resistant, only 1 isolate (5.3%) was resistant to CIP and SXT. None of the pulsotype C isolates was resistant to CIP and SXT, while none of the pulsotype D isolates was resistant to TET and SXT. Thus the antimicrobials isolates in these 4 pulsotypes having major differences in resistance to are CIP, GEN, SXT, and TET.

The distribution of these 4 pulsotypes in different years was compared to see if changes in their prevalence have occurred (Table 5). Isolates in pulsotype A accounted for 90.5% of the MRSA in 1998 (TSAR I) but it decreased to account for only 57.4% in 2004 (TSAR IV), while pulsotype D was not found in 1998 but comprised 20.4% (11/54) of the MRSA isolates causing HAI in 2004. The proportions of pulsotypes B and C also changed over the years. However, the most significant change was the decrease in pulsotype A and increase in pulsotype D. These changes in pulsotype distribution could account for the change in rates of antimicrobial resistance to CIP, TET and SXT, all of which decreased in 2002 and 2004. Isolates in pulsotypes B to D were nearly 100% susceptible to SXT. In addition, pulsotypes B and C isolates were almost all susceptible to CIP, while pulsotype D isolates were all susceptible to TET. These results indicated that the epidemiology of

MRSA in Taiwan hospitals is changing. However, more studies are needed to obtain a larger sample size. In addition, further studies are needed to determine the disease spectrum, risk factors, and patient demographics.

Differences in the molecular characteristics of the isolates in these 4 pulsotypes were also found. All pulsotype A isolates carry SCC*mec* type III and are either ST239 or ST241. Pulsotypes B, C, and D isolates carry the SCCmec types IV, V, and II, respectively. The major SCC*mec* types found in hospitals in other countries have mostly been either types II or III and ST239 and ST241 belong to the epidemic clones found in many hospitals in other parts of the world [25]. However MRSA isolates possessing SCC*mec* types IV and V have until recent years been mostly associated with community-onset infections in other countries.

It is interesting to note that both pulsotypes B and C isolates all belong to ST59. Data on the prevalence of SCC*mec* V is still limited and ST59 has been described infrequently. However, only pulsotype C isolates possess the Panton-Valentine leukocidin (PVL) gene. Production of the PVL cytotoxin was considered as one genetic marker for C-MRSA and PVL-positive MRSA have been associated not only with skin and soft tissue infections but also severe and fatal infections, such as necrotizing pneumonia. Interestingly, a longititudal study of MRSA isolates in San Francisco area found ST59-SCC*mec* IV MRSA increased steadily from 1999 to become one of the four major clones associated with community onset MRSA (C-MRSA) in recent years [30].

These results indicated that the epidemiology of MRSA causing hospital infections is changing in Taiwan and MRSA that used to be considered C-MRSA may be migrating into the hospital environment.

- **Extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae*.**

A total of 259 *E. coli* HAI isolates were collected in the 4 rounds of TSAR,

including 70, 33, 71, and 85 from 1998, 2000, 2002, and 2004, respectively. Among these 259 isolates, 79 met the screening criteria for possible ESBL producer. Of these 79 isolates, 33 tested positive for ESBL by using the CLSI confirmatory testing method. Thus among the *E. coli* isolates causing HAI between 1998 and 2004, 12.7% (33/259) were ESBL producers. The proportions of ESBL producers in the four rounds of TSAR increased from 8.6% (6/70) in 1998 to 14.1%. (12/85). The majority of these ESBL producing *E. coli* isolates were from urine (69.7%, 23/33) with a few from blood (12.1%, 4/33), respiratory (3.0%, 1/33), and wound (15.1%, 5/33).

For *K. pneumoniae*, there were a total of 190 HAI isolates collected in the 4 rounds of TSAR, including 63, 17, 49, and 61 isolates each from 1998, 2000, 2002, and 2004, respectively. Among these 190 HAI *K. pneumoniae* isolates, 65 met the screening criteria for possible ESBL producer. Of these 65 isolates, 50 were confirmed to be ESBL producers. Thus the ESBL rate was 26.3% (50/190) overall for *K. pneumoniae* isolates causing HAI between 1998 and 2004 in Taiwan hospitals. The proportions of ESBL producing *K. pneumoniae* in the four rounds of TSAR increased from 23.8% (15/63) in 1998 to 36.1%. (22/61). The majority of these ESBL producing *K. pneumoniae* isolates was from urine (36.0%, 18/50) and blood (28.0, 14/50), with the rest from respiratory and wounds (18% each, 9/50). Thus *K. pneumoniae* ESBL producers are more common cause of hospital acquired bacteremia than *E. coli*.

The significant increases in resistance of *E. coli* and *K. pneumoniae* to several β -lactam antimicrobials and fluoroquinolone ciprofloxacin observed (Table 2) can be explained by the increase of ESBL producing strains in these two species since ESBL producers are often associated with fluoroquinolone resistance. Further studies are needed to delineate the molecular epidemiology of these ESBL producers causing HAI to see if it is due to clonal spread or acquisition of resistance determinants.

■ Vancomycin resistant enterococci

Despite the high prevalence of MRSA, the overall rates of VRE has been at less than 1% in *E. faecalis* (0.8%, 8/1151) and at less than 8% (7.8%, 14/179) in *E. faecium* over the years. Among these, there were only 4 isolates of HAI vancomycin resistant *E. faecium* in the TSAR collection, all shared identical PFGE pattern (Figure 2) and were all from one hospital indicating possible outbreak at that hospital. These VRE isolates possessed the *vanA* resistance determinant by PCR.

(五)、Conclusions and Suggestions (結論與建議)

Using molecular typing techniques, we showed that the majority of MRSA causing hospital-acquired infections (HAI) in Taiwan belong to a closely related highly resistant clone (pulsotype A, ST239 or ST241:SCC*mec* III). Our data also revealed that the epidemiology of MRSA causing infections in Taiwan hospitals is changing, with other pulsotypes (C and D) being found in increasing frequency. This is accompanied by the decrease in resistance of HAI MRSA to ciprofloxacin, tetracycline, and SXT since the resistance pattern of pulsotypes C (ST59, SCC*mec* V) and D (ST5:SCC*mec* II) are distinct from that of pulsotype A. Panton Valentine leukocidin (PVL)-positive strains can cause wound infections as well as other potentially more serious life threatening infections. The finding of PVL-positive clones (pulsotype C) in HAI patients is a concern since it indicated that this virulent clone has migrated from the community to hospital environment.

Molecular typing performed in this study provides a basis for further characterization on the isolates in each pulsotype to identify virulence factors contributing to their persistence and propagation in Taiwan hospitals. Studies on disease spectrum and characteristics of the patients infected by isolates in each pulsotype will help to identify risk factors associated with each clone (pulsotype). Studies are also needed to identity if and how MRSA are being transmitted between the community and hospital environment. Understanding the roles these different clones play in MRSA epidemiology helps physicians in choosing the most appropriate treatment. Data from these studies can help to define management regime, infection control measures and intervention strategies to prevent the further transmission of MRSA in the hospitals and decrease its spread in the community also.

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（七）圖表 Tables and Figures.

Table 1A. Specimen source of 7 common bacterial species isolated from patients with hospital acquired infections in Taiwan*

Specimen type	*E. coli*						*K. pneumoniae*					
	I	II	III	IV	I to IV		I	II	III	IV	I to IV	
	N	N	N	N	Total N	%	N	N	N	N	Total N	%
Blood	12	15	15	13	55	21.2%	13	6	18	23	60	31.6%
Respiratory	6	2	5	4	17	6.6%	23	4	6	11	44	23.2%
Urine	38	14	38	52	142	54.8%	14	6	14	19	53	27.9%
Wound	9	1	9	10	29	11.2%	6	0	7	7	20	10.5%
Others	5	1	4	6	16	6.2%	7	1	4	1	13	6.8%
Total	70	33	71	85	259		63	17	49	61	190	

Specimen type	*A. baumannii*						*P. aeruginosa*					
	I	II	III	IV	I to IV		I	II	III	IV	I to IV	
	N	N	N	N	Total N	%	N	N	N	N	Total N	%
Blood	16	0	19	13	48	23.3%	13	7	16	9	45	15.3%
Respiratory	50	9	21	14	94	45.6%	63	11	18	17	109	36.9%
Urine	13	1	7	4	25	12.1%	28	2	26	21	77	26.1%
Wound	5	1	1	3	10	4.9%	17	4	5	5	31	10.5%
Others	18	2	3	6	29	14.1%	20	0	10	3	33	11.2%
Total	102	13	51	40	206		141	24	75	55	295	

Specimen type	*S. aureus*					
	I	II	III	IV	I to IV	
	N	N	N	N	Total N	%
Blood	27	29	28	34	118	38.9%
Respiratory	44	10	11	12	77	25.4%
Urine	3	1	4	3	11	3.6%
Wound	31	6	6	23	66	21.8%
Others	14	4	7	6	31	10.2%
Total	119	50	56	78	303	

Specimen type	*E. faecalis*						*E. faecium*					
	I	II	III	IV	I to IV		I	II	III	IV	I to IV	
	N	N	N	N	Total N	%	N	N	N	N	Total N	%
Blood	7	8	8	7	30	29.4%	0	1	3	3	7	21.2%
Respiratory	0	1	0	0	1	1.0%	0	0	0	0	0	0.0%
Urine	7	11	12	10	40	39.2%	1	7	6	4	18	54.5%
Wound	5	1	8	6	20	19.6%	1	1	2	2	6	18.2%
Others	2	1	0	8	11	10.8%	0	0	1	1	2	6.1%
Total	21	22	28	31	102		2	9	12	10	33	

* TSAR I (1998), TSAR II (2000), TSAR III (2002), TSAR IV (2004).

Table 1B. Specimen source of 7 common bacterial species isolated from patients after 3 days of hospitalization (excluding HAI isolates)*

Specimen type	*E. coli*					*K. pneumoniae*				
	II	III	IV	II to IV		II	III	IV	II to IV	
	N	N	N	Total N	%	N	N	N	Total N	%
Blood	14	20	92	126	26.6%	12	13	17	42	14.7%
Respiratory	12	15	19	46	9.7%	31	44	52	127	44.4%
Urine	40	23	90	153	32.3%	6	8	36	50	17.5%
Wound	17	32	52	101	21.4%	3	18	25	46	16.1%
Others	9	18	20	47	9.9%	6	7	8	21	7.3%
Total	92	108	273	473		58	90	138	286	

Specimen type	*A. baumannii*					*P. aeruginosa*				
	II	III	IV	II to IV		II	III	IV	II to IV	
	N	N	N	Total N	%	N	N	N	Total N	%
Blood	6	4	10	20	6.9%	6	5	10	21	3.7%
Respiratory	33	57	84	174	60.0%	91	85	148	324	57.1%
Urine	2	6	10	18	6.2%	11	22	32	65	11.5%
Wound	11	14	19	44	15.2%	29	27	54	110	19.4%
Others	6	14	14	34	11.7%	11	14	22	47	8.3%
Total	58	95	137	290		148	153	266	567	

Specimen type	*S. aureus*				
	II	III	IV	II to IV	
	N	N	N	Total N	%
Blood	19	26	29	74	15.1%
Respiratory	46	41	68	155	31.6%
Urine	2	4	4	10	2.0%
Wound	45	52	92	189	38.6%
Others	16	24	22	62	12.7%
Total	128	147	215	490	

Specimen type	*E. faecalis*					*E. faecium*				
	II	III	IV	I to IV		II	III	IV	I to IV	
	N	N	N	Total N	%	N	N	N	Total N	%
Blood	11	8	8	27	10.1%	1	5	2	8	13.3%
Respiratory	0	2	0	2	0.7%	1	0	0	1	1.7%
Urine	15	18	42	75	28.1%	7	7	13	27	45.0%
Wound	34	33	41	108	40.4%	4	1	3	8	13.3%
Others	12	18	25	55	20.6%	3	1	12	16	26.7%
Total	72	79	116	267		16	14	30	60	

* TSAR I (1998), TSAR II (2000), TSAR III (2002), TSAR IV (2004).

Table 2A. Prevalence of antimicrobial resistance in 6 common bacterial species isolated from patients with hospital acquired infections*

Antimicrobial Agent	*E. coli*					*K. pneumoniae*				
TSAR	I	II	III	IV	p^a	I	II	III	IV	*p*
No. of isolates	*70*	*33*	*71*	*85*		*63*	*17*	*49*	*61*	
Amikacin	2.9	3	9.9	7.1	NS	17.5	11.8	4.1	19.7	NS
Amoxicillin/Clavulanate	17.1	6.1	28.2	37.6	<0.01	12.7	11.8	12.2	31.1	<0.01
Ampicillin	85.7	75.8	91.5	89.4	NS	Intrinsic resistance				
Aztreonam	4.3	12.1	8.5	12.9	<0.01	15.9	11.8	10.2	27.9	0.09
Cefazolin	21.4	18.2	42.3	47.1	<0.01	28.6	29.4	24.5	47.5	0.03
Cefepime	NT	3	7	12.9	NS	NT	11.8	2	24.6	<0.01
Cefotaxime	NT	6.1	14.1	15.3	NS	NT	17.6	8.2	29.5	0.02
Cefoxitin	NT	6.1	22.5	35.3	<0.01	NT	5.9	8.2	27.9	0.01
Ceftazidime	2.9	9.1	8.5	22.4	<0.01	11.1	11.8	8.2	19.7	NS
Cefuroxime	15.7	15.2	35.2	44.7	<0.01	28.6	29.4	16.3	45.9	<0.01
Ciprofloxacin	22.1	27.3	46.5	41.2	<0.01	15	23.5	14.3	39.3	<0.01
Gentamicin	52.9	57.6	60.6	55.3	NS	31.7	35.3	20.4	39.3	NS
Imipenem	0	0	0	1.2	NS	0	0	0	1.6	NS
Trimethoprim/sulfa.(SXT)	75.7	78.8	83.1	68.2	NS	46	52.9	24.5	50.8	0.03

Antimicrobial Agent	*A. baumannii*					*P. aeruginosa*				
TSAR	I	II	III	IV	*p*	I	II	III	IV	*p*
No. of isolates	*102*	*13*	*51*	*40*		*141*	*24*	*75*	*55*	
Amikacin	61.8	76.9	60	61	NS	12.1	0	1.3	0	<0.01
Ampicillin/Sulbactam	NT	61.5	50	58.5	NS	NT	NT	NT	NT	
Aztreonam	86.3	92.3	66	82.9	0.02	10.7	12.5	17.3	10.9	NS
Cefepime	NT	38.5	36	36.6	NS	NT	12.5	5.3	5.5	NS
Ceftazidime	59.8	61.5	62	68.3	NS	11.3	20.8	13.3	12.7	NS
Ciprofloxacin	65.7	76.9	70	73.2	NS	12.9	12.5	16	12.7	NS
Gentamicin	80.4	84.6	70	75.6	NS	30.7	20.8	26.7	9.1	0.02
Imipenem	1	0	2	12.2	<0.01	7.1	8.3	6.7	10.9	NS
Levofloxacin	NT	NT	36	56.1	0.09	NT	NT	17.3	12.7	NS
Piperacillin/Tazobactam	NT	69.2	38	68.3	<0.01	NT	16.7	14.7	10.9	NS
Ticarcillin/Clavulanate	54.9	69.2	36	63.4	0.03	27.7	20.8	24	20	NS

Antimicrobial Agent	*E. faecalis*	*E. faecium*
TSAR	I to IV combined*	I to IV combined*
No. of isolates	*102*	*33*
Ampicillin	2.5	87.1
Chloramphenicol	41.6	12.1
Erythromycin	90.9	97.0
Ciprofloxacin	22.2	75.8
Gentamicin-High Level	78.4	81.8
Penicillin	2.0	90.9
Rifampin	32.1	87.1
Tetracycline	90.1	33.3
Vancomycin	0	12.1

* Data from TSAR, Taiwan Surveillance of Antimicrobial Resistance; 1998 (I), 2000 (II), 2002 (III), 2004 (IV). *p* value from χ^2 for trend in proportions analysis; NS, not significant (p >0.05). For *Enterococcus* spp., due to small number of HAI isolates, analysis was done with TSAR I-IV combined.

Table 2B. Prevalence of antimicrobial resistance in 6 common bacterial species isolated from patients after 3 days of hospitalization (excluding HAI isolates)*

Antimicrobial Agent	*E. coli*				*K. pneumoniae*			
TSAR	II	III	IV	p^a	II	III	IV	*p*
No. of isolates	*92*	*108*	*213*		*58*	*90*	*138*	
Amikacin	6.5	8.3	4.7	NS	19.0	7.8	13.0	NS
Amoxicillin/Clavulanate	12.0	20.4	27.2	0.01	1.7	10.0	19.6	<0.01
Ampicillin	85.9	88.9	82.6	NS				
Aztreonam	7.6	7.4	10.8	NS	13.8	5.6	19.6	0.01
Cefazolin	28.3	32.4	38.5	NS	32.8	24.4	34.8	NS
Cefepime	5.4	5.6	8.5	NS	10.3	2.2	13.0	0.02
Cefotaxime	10.9	12.0	12.2	NS	19.0	7.8	18.8	0.05
Cefoxitin	13.0	15.7	21.6	NS	3.4	8.9	23.2	<0.01
Ceftazidime	2.2	9.3	12.2	0.02	13.8	10.0	15.2	NS
Cefuroxime	21.7	25.9	27.7	NS	29.3	16.7	30.4	0.05
Ciprofloxacin	17.4	30.6	36.6	<0.01	5.2	13.3	27.5	<0.01
Gentamicin	43.5	45.4	44.6	NS	31.0	20.0	26.8	NS
Imipenem	0.0	0.0	0.0	-	0.0	0.0	0.0	-
Trimethoprim/sulfa.(SXT)	76.1	62.0	70.9	NS	44.8	27.8	36.2	NS

Antimicrobial Agent	*A. baumannii*				*P. aeruginosa*			
TSAR	II	III	IV	*p*	II	III	IV	*p*
No. of isolates	*58*	*95*	*137*		*148*	*153*	*266*	
Amikacin	63.8	70.5	71.5	NS	9.5	2.0	1.1	<0.01
Ampicillin/Sulbactam	51.7	55.8	62.0	NS	NT	NT	NT	-
Aztreonam	87.9	86.3	86.1	NS	16.9	11.1	18.4	NS
Cefepime	32.8	32.6	47.4	0.04	11.5	5.2	9.0	NS
Ceftazidime	60.3	70.5	75.2	NS	16.9	6.5	15.0	0.02
Ciprofloxacin	74.1	71.6	78.8	NS	14.2	12.4	13.9	NS
Gentamicin	82.8	80.0	81.0	NS	27.7	22.9	15.0	<0.01
Imipenem	0.0	3.2	18.2	<0.01	6.8	3.3	10.9	0.02
Levofloxacin		33.7	59.1	<0.01	NT	12.4	13.9	NS
Piperacillin/Tazobactam	58.6	45.3	69.3	<0.01	12.8	8.5	13.9	NS
Ticarcillin/Clavulanate	65.5	45.3	62.0	0.01	25.7	17.6	27.4	NS

Antimicrobial Agent	*E. faealis*				*E. faecium*
TSAR	II	III	IV	*p*	II to IV combined*
No. of isolates	*72*	*79*	*116*		*60*
Ampicillin	2.8	0.0	0.0	NS	83.3
Chloramphenicol	40.3	41.8	31.4	NS	5.0
Erythromycin	76.4	67.1	68.6	NS	88.3
Ciprofloxacin	12.5	6.3	10.2	NS	71.7
Gentamicin-High Level	66.7	55.7	50.4	NS	75.0
Penicillin	2.8	0.0	0.7	NS	85.0
Rifampin	25.0	43.0	36.5	0.03	91.7
Tetracycline	81.9	82.3	76.6	NS	40.0
Vancomycin	0.0	0.0	0.0	-	5.0

*Insufficient information (No admission date) to calculate TSAR I data for this analysis. Due to small number of isolates in each round of survey, analysis was done with TSAR I-IV combined for *E. faecium*. *p* value from c2 for trend in proportions analysis; NS, not significant (p >0.05)

Table 3A. Prevalence of antimicrobial resistance among *S. aureus* and methicillin resistant *S. aureus* (MRSA) isolated from patients with hospital acquired infections

Antimicrobial Agent	*S. aureus*					MRSA				
TSAR	I	II	III	IV	*p*	I	II	III	IV	*p*
No. of isolates	*119*	*50*	*56*	*78*		*95*	*39*	*38*	*54*	
Chloramphenicol	34.3	28	26.8	24.4	NS	42.1	30.8	28.9	25.9	NS
Ciprofloxacin	76.5	68	53.6	55.1	<0.01	94.7	84.6	76.3	77.8	<0.01
Clindamycin	77.3	68	69.6	69.2	NS	94.7	87.2	94.7	94.4	NS
Erythromycin	84	82	73.2	79.5	NS	100	100	100	100	NS
Gentamicin	79.6	70	66.1	67.9	NS	98.9	87.2	92.1	88.9	0.03
Oxacillin	79.8	78	67.9	73.1	NS	100	100	100	100	NS
Penicillin G	96.6	100	100	98.7	NS	100	100	100	100	NS
Tetracycline	85.7	86	69.6	57.7	<0.01	97.9	97.4	84.2	66.7	<0.01
Trimeth/Sulfa. (SXT)	71.4	66	48.2	41	<0.01	89.5	89.7	68.4	57.4	<0.01
Vancomycin	0	0	0	0	-	0	0	0	0	-

[#] Data from TSAR, Taiwan Surveillance of Antimicrobial Resistance; 1998 (I), 2000 (II), 2002 (III), 2004 (IV). *p* value from χ^2 for trend in proportions analysis; NS, not significant (p >0.05).

Table 3B. Prevalence of antimicrobial resistance among *S. aureus* and methicillin resistant *S. aureus* (MRSA) isolated from patients after 3 days of hospitalization (excluding HAI isolates).

Antimicrobial Agent	*S. aureus*				MRSA			
TSAR	II	III	IV	*p*	II	III	IV	*p*
No. of isolates	*130*	*149*	*215*		*96*	*104*	*158*	
Chloramphenicol	14.6	15.4	20.5	NS	15.6	17.3	22.2	NS
Ciprofloxacin	66.2	55.7	56.3	NS	89.6	79.8	76.6	0.04
Clindamycin	68.5	69.1	67.9	NS	88.5	93.3	86.7	NS
Erythromycin	82.3	74.5	77.2	NS	99.0	98.1	96.2	NS
Gentamicin	73.1	62.4	62.3	NS	97.9	88.5	84.2	<0.01
Oxacillin	73.8	69.8	73.5	NS	100.0	100.0	100.0	-
Penicillin G	99.2	98.7	98.1	NS	100.0	100.0	100.0	-
Tetracycline	77.7	75.2	60.0	<0.01	92.7	88.5	67.7	<0.01
Trimeth/Sulfa. (SXT)	66.9	51.0	40.5	<0.01	90.6	73.1	55.1	<0.01
Vancomycin	0.0	0.0	0.0	-	0.0	0.0	0.0	-

*Insufficient information (No admission date) to calculate TSAR I data for this analysis.

Table 4. Comparison of characteristics of the main pulsotypes of MRSA causing hospital acquired infections in Taiwan*

Characteristics	Pulsotype			
	A (n=174)	B (n=19)	C (n=9)	D (n=15)
n (%) resistant to:				
Chloramphenicol	46 (26.4)	18 (94.7)	9 (100)	0[#]
Ciprofloxacin	174 (100)	1 (5.3)	0	15 (100)
Clindamycin	162 (93.1)	19 (100)	9 (100)	15 (100)
Erythromycin	174 (100)	19 (100)	9 (100)	15 (100)
Gentamicin	173 (99.4)	15 (78.9)	1 (11.1)	15 (100)
Tetracycline	174 (100)	11 (57.9)	5 (55.6)	0
Trimeth/Sulfa. (SXT)	169 (97.1)	1 (5.3)	0	0
Molecular typing:				
SCC*mec* type	III	IV	V	II
Sequence type (# of ST/# tested)	ST239 (11/19)[§], ST241 (6/19),	ST59 (6/6)	ST59 (1)	ST5 (3/3)
PVL toxin gene	-	-	+	-

*Antimicrobial susceptibility testing, SCC*mec* typing and Panton-Valentine leukocidin (PVL) gene detection were done on all isolates. MLST to determine the sequence type (ST) was performed on selected isolates from each representative pulsotype.

[#] Although none of the 15 pulsotype D isolates were resistant to chloramphenicol, 10 of them had only intermediate susceptibility to it.

[§] 2 other isolates had ST239-like sequence type.

Table 5. Distribution of major pulsotypes in MRSA causing hospital acquired infections in different years

Pulsotype*	Number of isolates (%) with pulsotype in:				*P*
	1998 (n = 95)	2000 (n = 39)	2002 (n = 38)	2004 (n = 54)	
A	86 (90.5)	31 (79.5)	26 (68.4)	31 (57.4)	<0.001
B	3 (3.2)	4 (10.3)	7 (18.4)	5 (9.3)	0.03
C	0	2 (5.1)	1 (2.6)	6 (11.1)	0.01
D	0	1 (2.6)	3 (7.9)	11 (20.4)	<0.001

* See Figure 1 for pulsotype designation.

Figure 1. Dendrogram generated from cluster analysis of *Sma*I digested pulsed field gel electrophoresis (PFGE) patterns of 226 MRSA isolates causing hospital-acquired infections in Taiwan. Only one isolate of a pulsotype is shown with the number of isolates in that type listed (No.). SCC*mec* type and sequence type (ST) results are also shown

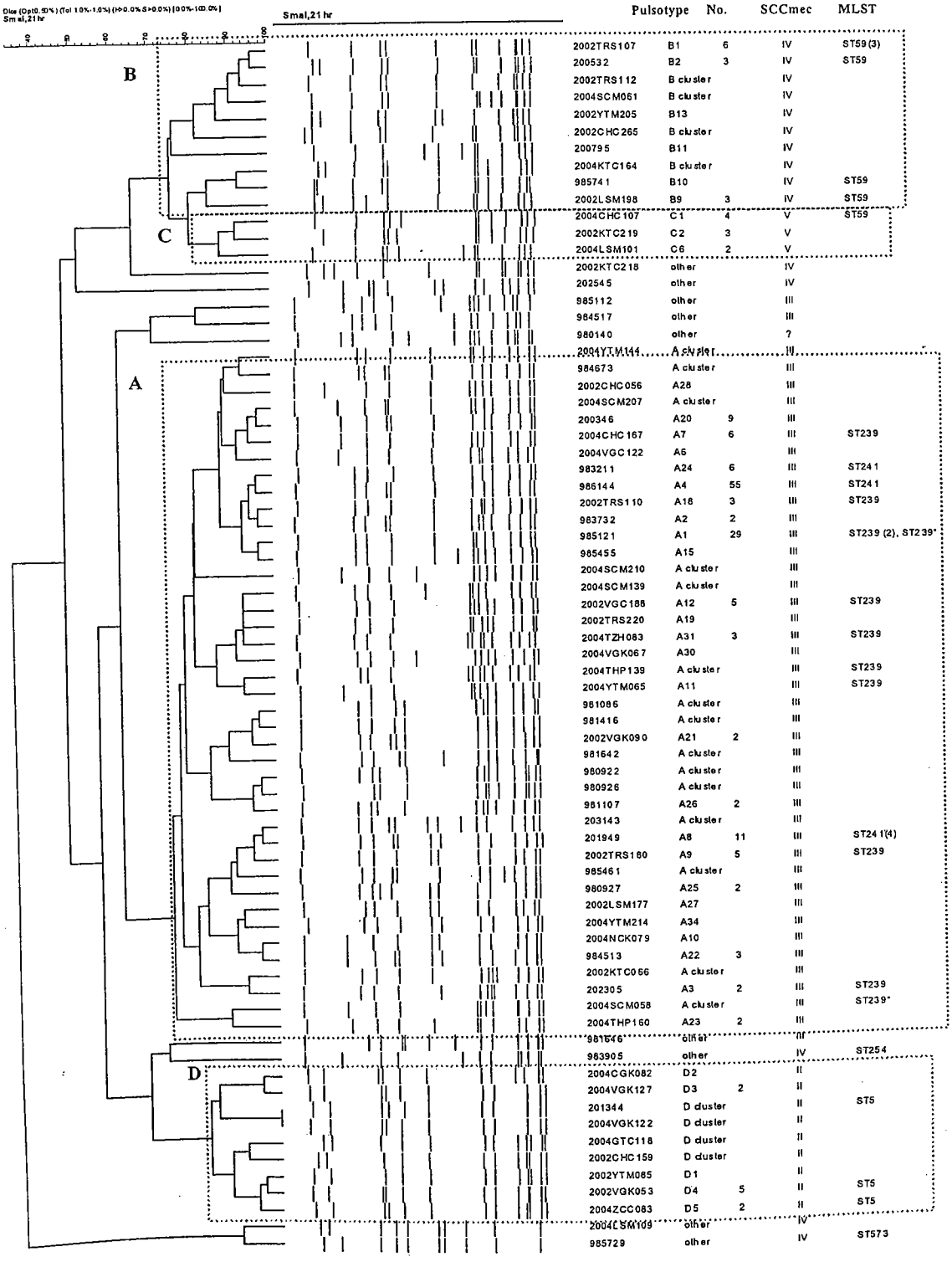
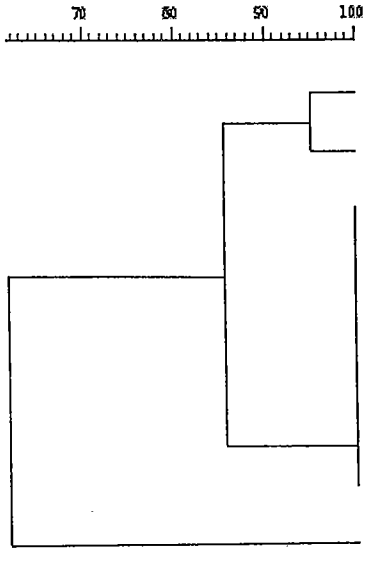


Figure 2. Dendrogram of 9 vancomycin resistant *Enterococcus faecium*. Four of the isolates were from hospital acquired infection (HAI) patients. The hospitals where the isolates were collected from are listed.



	HAI	Hosp.
203620		TRS
203627	Y	TRS
203005	Y	PHL
203602	Y	TRS
203603		TRS
203622		TRS
203628		TRS
203635	Y	TRS
980614		TRS
		GTC