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行政院衛生署疾病管制局九十八年度科技研究發展計畫

院內感染多重抗藥菌分子特性及流行病學

Molecular characterization and epidemiology of multidrug resistant nosocomial bacteria in Taiwan

研 究 報 告

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一、 摘要

（一）中文摘要

多重抗藥菌造成病人可因治療失敗，病情加重及住院日之加長，而增加病人及國家醫療體系之負擔，病患亦可因治療無效而死亡。近年來細菌多重抗藥性之持續上升，使醫院從廣泛性抗生素至後線抗生素使用都逐年增加，加上住院病人長期侵入性醫療器療(如：呼吸器或導尿管)之使用，亦增加多重抗藥菌寄生于病人體內不同部位之機會，進而俟機侵入引起院內感染(hospital-acquired or nosocomial infections)，甚至造成群突發案(outbreak)，故引起院內感染的細菌的抗藥性最嚴重。而後線抗生素之使用增加，亦引起對後線抗生素感受性降低及具抗藥性菌之逐漸浮現，故進行此計畫，研究院內感染常見之多重抗藥菌，及對新興及不尋常抗藥菌進行調查。

此計畫使用國家衛生研究院感染症研究組之「全國微生物抗藥性監測計劃(Taiwan Surveillance of Antimicrobial Resistance,簡稱 TSAR)」不同年度之菌種，進行抗藥基因型及分子流行病學之研究，介此了解多重抗藥菌之抗藥機制及菌種演變、分子流行病學及其於不同醫院之分佈情況。TSAR 每兩年進行一次，自第三期(2002 年)起之 TSAR 醫院為相同的 26 家醫院，包含分佈於北、中、南、東部之 11 家醫學中心及 15 家區域醫院；每期收菌期間為三個月(七至九月)，收集醫院微生物實驗室判斷為具臨床意義之菌，於國衛院進行抗藥測試。每家醫院各收住院病人非加護病房菌株 100 株、加護病房菌株(成人)30 株、及小兒科菌株 20 株，並再多收血液菌株 20 株(第六期 TSAR 則增加至 50 株無菌部位菌種)及 10 株腸球菌。並請醫院提供所收集菌株之相關資料，包含檢體、採檢日、住院日、病歷號碼、生日或年齡，亦請感控加註是否為院內感染菌株。

此計劃今年調查了第六期 TSAR VI (2008)之抗藥菌包含：抗甲氧苯色葡萄球菌(MRSA)、對 carbapenem 具抗藥性之綠膿桿菌及鮑氏不動桿菌(Carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ,CRPA 及 CRAB)、對廣泛性乙內醯氨感受性降低及具抗藥性的大腸桿菌(*Escherichia coli*)及克雷白氏肺炎桿菌(*Klebsiella pneumoniae*)、及對萬古黴素具抗藥性之腸球菌(VRE)，進行抗藥基因型及分子流行病學之研究，以上之菌多為院感菌，但 VRE 亦包含非院感菌。此計畫亦開始偵測新興抗藥菌，今年調查了對萬古黴素感受性降低或具混合抗藥性的 MRSA (Vancomycin reduced susceptible or heterogeneous vancomycin intermediate-resistant *S. aureus*, hVISA)。為了解 MRSA 不同基因型菌種所造成的疾病、嚴重性、及治療選擇之差異，今年亦設計問卷以便更進一步了解其臨床流行病學。此外，為能較即時偵測新興抗藥菌之產生，亦於今年底起對六家 TSAR 醫院進行指標多重抗藥菌之收集，以便於明年初進一步測試。今年並技術協助，調查 TSAR 醫院之群突發案件及不尋常抗藥菌。

MRSA 佔 2008 年院感 *S. aureus* 之 69%，這些 MRSA(68 株)仍屬於四個主要菌群，其中以抗藥性最高的 pulsotype A 及 D 型為主，各佔 58.8%及 14.7%；pulsotype A 對 ciprofloxacin (CIP)、gentamicin(GEN)、tetracycline 及 trimethoprim/sulfamethoxazole (SXT)幾乎全具抗藥性，pulsotype D 則全對 CIP 及 GEN 具抗性。另外，對 CIP 及 SXT 幾乎全無抗藥性之 pulsotypes B 及 C，各佔了約 9%，此四種菌群以成為台灣醫院之最常見 MRSA。今年並挑選了 35 株 vancomyciny 最小抑制濃度(MIC)為 2 μg/mL 之 MRSA，測試對 vancomycin 有混合抗藥性之菌(hVISA)，發現其中有 8 株符合 hVISA 條件，這些菌需特殊實驗方法測試，一般微生物實驗室 routine 方法會偵測不出，是須注意的新興抗藥菌。今年亦對一家區域醫院進行 ICU 病人之 MRSA 帶菌率調查，發現有近 23%的病人於住院期間帶有 MRSA。為了解 MRSA 同基因型菌種所造成的疾病、嚴重性、及治療選擇之差異，及 hVISA 是否因病人使用 vancomycin 而浮現，現正進行問卷調查。

對廣泛性乙內醯氨具抗藥性(extended spectrum β-lactam non-susceptible，簡稱 ESβ –NS)的大腸桿菌及克雷白氏肺炎桿菌(*E. coli* and *K. pneumoniae*)各佔院感 *E. coli* 及 *K. pneumoniae* 菌之 48% (60 株)及 44% (38 株)。調查結果顯示，AmpC ß-lactamase 已是 ESβ -NS *E. coli* 之主要抗藥機制，bla_{AmpC} 之基因可從 90%之菌中測到，包含 31.7%同時帶有 bla_{ESBL} 及 bla_{AmpC} 抗藥基因，而單帶有 bla_{ESBL} 之菌只佔 8.3%。但 bla_{ESBL} 仍是 ESβ –NS *K. pneumoniae* 之主要抗藥機制，佔 65.8%，包含 18.4%同時帶有 bla_{ESBL} 及 bla_{AmpC} 抗藥基因，而單帶有 bla_{AmpC} 之菌亦佔 18.4%。在此兩種菌 CTX-M 類 ESBL 都是主要的 bla_{ESBL}，但 CMY 類是 ESβ -NS *E. coli* 中主要的 bla_{AmpC}，而 DHA 類是 ESβ -NS *K. pneumoniae* 中主要的 bla_{AmpC}。過去之研究已知這些抗藥基因多位於多重抗藥質體上，而脈衝電泳法(PFGE)亦顯示，這些院感 ESβ-NS *E. coli* 及 *K. pneumoniae* 菌間親聯性大多不高，除少數幾株從同一家醫院來的菌有一樣 pattern。總和這些結果顯示，這些抗藥菌主要是經由抗藥基因在不同菌株間轉移而擴散。

台灣醫學界面臨的一個重要感染控制挑戰是，carbapenem-resistant 包氏不動桿菌(CRAB)。TSAR 資料顯示，在台灣醫院從加護病房分離出的包氏不動桿菌中，CRAB 在 2004 年佔 19%，在 2006 年為 44.7%，在 2008 年已高到 68.8%。從院感個案分離出的包氏不動桿菌中，CRAB 在 2004 年佔 12%，在 2006 年為 34%，在 2008 年高到 51%。這些 CRAB 大多（75%）來自 70 歲以上老年病人，表示台灣醫療機構中 CRAB 可能集中在某特定患者群。對 carbapenem 之抗藥機制在 CRAB 是 IS*Aba1* 跟 bla_{OXA} 之關連，且 2008 年 33 株院感 CRAB 菌中，有一包含 12 株來自 10 家醫院的 cluster(≥80%相似性)，值得更進一步探討。

另一個需注意的是抗萬古黴素腸球菌(Vancomycin resistant enterococci, VRE)。TSAR VI 資料顯示，Vancomycin resistant *E. faecium* 從 2004 年之 4.1%及 2006 年之 11.7%，於 2008 年上升至 25%(29/116)，其中有五株是院感個案，但其他有 16 株菌來自病人住院三天以後才分離出的。所有的 29 株 2008 年 VRE 中，有三對有一樣的 PFGE

pattern，這三對 VRE 有兩對來自一家醫院四個病人，另一對來自另一家醫院兩個不同病人，而雖然其他 VRE 之 PFGE pattern 顯示其親聯性不高，但因 2008 年之 VRE 來自不同地區的 11 家醫院，加上其明顯增加($p < 0.05$)，所以需增加對 VRE 之監控。

此計劃比較不同年度菌種之抗藥機制及基因型，以長期追蹤國內多重抗藥菌之演變，協助建立多重抗藥菌之基因與流行病學資料，並介此了解這些多重抗藥菌在國內不同醫院之分佈情況，做為制定防止抗藥菌擴散及衍生之參考資料。此計畫並建立了 MRSA 分子流行病學之國際標準測試平台，可用於跟不同國家地區盛行之 MRSA 比對，以偵測 MRSA 之國際擴散。此計畫今年亦協助兩件 CRAB 群突發之實驗調查，使醫院得即時控制其擴散。控制院內感染之發生，不但能減少醫療費用，且可增進民眾之健康，對醫院、病人、和國家公衛機構都有貢獻。

關鍵詞：院內感染、多重抗藥菌、抗甲氧苯青黴素金黃色葡萄球菌、抗廣泛性乙內醯氨大腸桿菌、抗廣泛性乙內醯氨克雷白氏肺炎桿菌、抗 carbapenem 鮑氏不動桿菌、抗萬古黴素腸球菌。

英文摘要

Background and Purpose

Bacterial pathogens causing nosocomial infections have the highest rates of antimicrobial resistance. They are also resistant to multiple classes of antibiotics, which compromise treatment options and outcome. Strategies for the control of multidrug resistant bacteria require understanding of the mechanisms of resistance and local epidemiology within a region. This project utilized isolates collected different rounds of the Taiwan Surveillance of Antimicrobial Resistance (TSAR) project. TSAR is conducted by the National Health Research Institutes (NHRI) every 2 years. The same 26 hospitals from the 4 regions of Taiwan have participated in TSAR since 2002. This year we studied mostly nosocomial isolates from TSAR VI (2008), including 68 methicillin resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactam non-susceptible *Escherichia coli* (60 isolates) and *Klebsiella pneumoniae* (38 isolates), carbapenem-resistant *Acinetobacter baumannii* (33 isolates), and vancomycin-resistant *Enterococcus faecium* (VREfm, 29 isolates). Phenotypic and molecular characterizations were performed to investigate the mechanisms of resistance and to determine clonal relatedness. This year we also assisted in two investigations of CRAB outbreaks in 2 hospitals.

Methods

Nosocomial *E. coli* and *K. pneumoniae* having aztreonam, cefotaxime, or ceftazidime minimum inhibitory concentrations > 1 μ g/ml were subjected to extended spectrum β-lactamase (ESBL) confirmatory test and AmpC β-lactamase detection by modified Hodge test, Disk Potentiation test and Double Disc Synergy test. Multiplex PCR was used to detect the genes encoding ESBL (SHV, TEM, CTX-M type and others), and plasmid-mediated class C AmpC β-lactamases (DHA, CMY, and other types). The genes detected were selected for sequencing to confirm their identity. For MRSA, multiplex PCR was also used to determine the types of staphylococcus cassette chromosome *mec* (*SCCmec*), the element responsible for methicillin resistance and its mobilization. The presence of Panton-Valentine leukocidin (PVL) toxin genes in MRSA was determined by PCR. Multilocus sequence typing (MLST) (by PCR and DNA sequencing) was performed on selected representative strains from each major pulsotype to determine their sequence type (ST). Modified population analysis profile (PAP) was used to detect MRSA with heterogeneous vancomycin-intermediate resistance (hVISA). The *van*A and *van*B vancomycin resistance determinants were determined on VREfm. The presence of genes encoding carbapenemase and their association with ISAba1 was investigated by PCR and sequencing on CRAB. Pulsed Field Gel Electrophoresis (PFGE) was used to determine strain relatedness for all organisms studied to look for clonal spread.

Results

Among the nosocomial *S. aureus* from TSAR VI (2008), 69% (68 isolates) were MRSA. These MRSA belong to 4 major clones. The most resistant 2 clones, pulsotype A and D, comprised 58.8% and 14.7% of the 2008 nosocomial MRSA, respectively. Pulsotype A isolates are nearly all resistant to ciprofloxacin (CIP), gentamicin (GEN), tetracycline (TET) and trimethoprim/sulfamethoxazole (SXT), while pulsotype D are susceptible to TET and SXT. Pulsotypes C and D each comprised 8.9% of the nosocomial MRSA in TSAR VI, indicating they have also established a presence in Taiwan hospitals even though they are nearly all susceptible to CIP and SXT. Pulsotype C is unique in Taiwan and rarely found in other countries. This year we also investigated 35 MRSA isolates with vancomycin MIC of 2 μg/mL to look for strains with heterogeneous vancomycin-intermediate resistance (hVISA). Eight hVISA was detected. Further genotypic characterization of these hVISA will be carried out. A pre-designed questionnaire is being used to investigate the clinical presentation, risk factors, disease spectrum and treatment outcomes of the 4 major MRSA clones as well as the hVIST strains.

A total of 48% (60 isolates) and 44% (38 isolates) of the nosocomial *E. coli* and *K. pneumoniae* isolates in TSAR VI (2008) had reduced susceptibility and resistance to extended spectrum ß-lactams (ESß-NS). In ESß-NS *E. coli*, 90% of the isolates carried AmpC ß-lactamase genes (bla_{AmpC}) including 31.7% also co-carried ESBL ß-lactamase genes (bla_{ESBL}), while only 8.3% were found to carry just bla_{ESBL}. In ESß-NS *K. pneumoniae*, bla_{ESBL} still predominated, with 65.8% being bla_{ESBL}carriers including 18.4% also co-carried bla_{AmpC}, while another 18.4% carried just bla_{AmpC}. The predominant bla_{ESBL} was CTX-M type for both species. CMY-type bla_{AmpC} predominated in *E. coli*, while DHA-type bla_{AmpC} predominated in *K. pneumoniae*. PFGE revealed that except a few isolates from the same hospitals having indistinguishable PFGE pattern, most are distinct from each other. Since the bla_{ESB}L and bla_{AmpC} genes detected have been well established to be carried on plasmids, these results indicate the continued transfer of resistant plasmid among different strains of bacteria and clonal spread may play a role in intra-hospital spread of these resistant organisms. The difference in bla_{ESBL} and bla_{AmpC} distribution among these 2 species warrant further investigation.

A major infection control challenge facing the medical community in Taiwan is carbapenem resistant *A. baumannii* (CRAB). CRAB has escalated to an alarming high rate in Taiwan hospitals. Among *A. baumannii* from ICUs in TSAR, CRAB was 19% (20/105) in

2004, 44.7% (46/103) in 2006, then further increased to 68.8% (86/125) in 2008. In 2002, 12.2% (5/14) of the HAI *A. baumannii* were CRAB. That rate increased to 33.8% (23/68) in 2006, and reached 50.8% (33/65) in 2008. The majority (75%) of CRAB were from elderly patients (>70 yo), indicating that CRAB may be concentrated in specific patient populations. Resistance in CRAB is mostly associated with the presence of IS*Aba1* upstream of the bla_{OXA}. PFGE revealed one cluster of 12 isolates from 10 hospitals sharing >80% similarity in PFGE patterns. Further investigations are needed on this cluster.

Another noteworthy increase in resistance is vancomycin-resistant enterococci (VRE). Although the prevalence of vancomycin resistant *Enterococcus faecalis* (VREfa) has remained at <1.0% in the past few years including 2008, there was significant increase in vancomycin resistant *E. faecium* (VREfm) in 2008. Among the 116 *E. faecium* in 2008, 25% (29 isolates) were vancomycin-resistant, which was a significant increase ($p < 0.05$) from 4.2% in 2004 (3/71) and 11.7% (9/77) in 2006. These 29 VREfm in 2008 were from 11 hospitals but 16 isolates were from 2 hospitals (9 and 7 isolates each). Among the 26 isolates from inpatients, only 5 were determined to be HAI by the hospitals but another 17 were from patients hospitalized for more than 2 days (3 to 58 days), thus most of these VREfm may also be from HAI. There were 3 pairs of VREfm with identical PFGE patterns, with each pair having distinct pattern from the other pairs, 2 pairs from 4 patients were from one hospital, and the other pair was from another hospital. These results indicated that some intra-hospital spread may have occurred as well as emergence of VRE in different hospitals.

Conclusions

Multidrug-resistant bacteria are prevalent Taiwan hospitals. Rates of carbapenem-resistance in *A. baumannii* from ICU and nosocomial isolates are approaching those of MRSA in Taiwan hospitals. MRSA in Taiwan comprised of 4 major clones. These clones have become endemic in Taiwan hospitals. MRSA with heterogeneous vancomycin-intermediate resistance is an emerging resistance to watch out for. Resistance to extended spectrum ß-lactams (ESß) in *E. coli* and *K. pneumoniae* has reached near 50%. Resistance in ESß *E. coli* is now mainly due to AmpC ß-lactamase but isolates co-carrying ESBL and AmpC ß-lactamases are also becoming common. ESBL ß-lactamases are still the major mechanisms of resistance to extended spectrum ß-lactams in *K. pneumoniae* but AmpC ß-lactamases are also prevalent. Significant increase in VRE in Taiwan hospitals was noted in 2008 and careful monitoring of this emerging resistance is needed to prevent their further spread. There is some evidence of intra-hospital spread of some of these resistant bacteria. This year we also assisted in investigation of 2 carbapenem-resistant *A. baumannii* outbreaks. Because TSAR is conducted every 2 years, we have also started a collection of selected

resistant bacteria from 6 TSAR hospitals to increase the timely detection of resistant bacteria. Testing of these isolates will be started early next year. Implementation of stringent infection control and antibiotic stewardship intervention measures are needed.

Key Words: Nosocomial infection, multidrug resistant organism, methicillin resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, extended-spectrum β-lactam resistant *Escherichia coli*, extended-spectrum β-lactam resistant *Klebsiella pneumoniae*, carbapenem resistant Acinetobacter baumannii, vancomycin-resistant enterococci (VRE).

本文

(一) 前言 (Background)

對抗生素具抗藥性之細菌，尤其是多重抗藥菌，近年來在世界各國持續增加，台灣亦如此，細菌抗藥性是一全球之公衛危機。病人可因感染抗藥菌而治療無效，導致病情加重及住院日之加長，且需使用昂貴之後線藥，而增加許多醫療費用和病患死亡率，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。近年來細菌多重抗藥性之持續上升，使醫院從廣泛性抗生素至後線抗生素使用都逐年增加，加上住院病人長期侵入性醫療器療(如：呼吸器或導尿管)之使用，亦增加多重抗藥菌寄生于病人體內不同部位之機會，進而俟機侵入引起院內感染(hospital-acquired or nosocomial infections)，甚至造成群突發案(outbreak)，故引起院內感染的細菌的抗藥性最嚴重。

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，可協助於制定防止抗藥菌之進一步擴散及衍生策略。比較不同年度所收集之菌種之抗藥機制及分子與臨床流行病學，亦可增加對抗藥菌演變之了解。國家衛生研究院感染症研究組於 1998 年即開始進行「台灣微生物抗藥性監測計畫(Taiwan Surveillance of Antimicrobial Resistance, 簡稱 TSAR)」，每兩年進行一次，監測對象為台灣北中南東地區之醫學中心及區域醫院病人所分離出的病原細菌。自第三期起之 TSAR 醫院皆為相同之 26 家醫院，包含 11 家醫學中心及 15 家區域醫院；收菌期段亦維持於七至九月。每家醫院各收住院病人非加護病房菌株 100 株、加護病房菌株(成人)30 株、及小兒科菌株 20 株，並再多收血液菌株 20 株(第六期 TSAR 則增加至 50 株無菌部位菌種)及 10 株腸球菌，於國衛院進行抗藥測試。並請醫院提供所收集菌株之相關資料，包含檢體、採檢日、住院日、病歷號碼、生日或年齡，亦請感控加註是否為院內感染菌株。第六期 TSAR 於今年前半年完成抗藥測試。

TSAR 資料顯示，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。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，又以對廣泛性乙內醯氨具抗性之大腸桿菌及克雷白氏肺炎桿菌(extended spectrum β-lactam resistant *E. coli* & *K. pneumoniae*)、對 carbapenem (imipenem or meropenem)具抗性的綠膿桿菌及鮑氏不動桿菌(carbapenem resistant *P. aeruginosa* and *A. baumannii*)、對甲氧苯青黴素具抗性的金黃色葡萄球菌(MRSA)、及對萬古黴素具抗性之腸球菌(Vancomycin resistant *E. faecalis* 及 *E. faecium*)之多重抗藥性最嚴重。

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，或將抗生素排出或阻止抗生素進入細菌內，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或質體(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[13,18]。

對廣泛性乙內醯氨感受性降低及具抗性之腸桿菌及克雷白氏肺炎桿菌(extended spectrum β-lactam resistant and reduced susceptible *E. coli* and *K. pneumoniae*)之主要抗藥機制為產生分解酵素，其中以質體誘導(plasmid-mediated)之廣泛性乙內醯氨酶(extended-spectrum β-lactamase; ESBL)及AmpC乙內醯氨酶(AmpC β-lactamase)最另人憂心，因這些抗藥基因較容易於不同細菌間傳遞。產生ESBL(ESBL producer)之菌大多在腸細菌科菌種 (Enterobacteriaceae)，具ESBL 的大腸桿菌及克雷白氏肺炎桿菌(ESBL producing *E. coli* & *K. pneumoniae*)對所有青黴素類、頭孢子菌素類及aztreonam抗生素均具抗藥性，但會被乙內醯氨酶抑制劑(β-lactamase inhibitor)抑制，故對內醯氨加乙內醯氨酶抑制劑合併藥(β-lactam/β-lactamase inhibitor combination)無抗藥性。而AmpC β-lactamase 除了對不同頭孢子菌素類抗生素有抗藥性，對乙內醯氨酶抑制劑(β-lactamase inhibitor)亦具抗性，但不一定對所有後線頭孢子菌素類及aztreonam具抗性。因為帶ESBL及AmpC β-lactamase之質體上大多同時帶有其他抗藥基因，故這些菌都是多重抗藥菌，導致醫生常以carbapenem 類最後線抗生素治療。但國內已有醫院發現對carbapenem有抗藥性的大腸桿菌，這也是一需仔細監測的新興抗藥菌。

金黃色葡萄球菌中對 methicillin 有抗藥性(methicillin-resistant *Staphylococcus aureus*, MRSA)之產生是經金黃色葡萄球菌獲得一移動性的基因片段，稱為 staphylococcal cassette chromosome mec (SCC*mec*)，而對 methicillin 產生抗性。MRSA 可製造一種對 β-lactam 類抗生素之親和性都降低的蛋白質，導致 MRSA 對 β-lactam 類抗生素幾乎全具抗性，包含所有青黴素類及頭孢子素類抗生素。同時，大多 MRSA 菌對不同非 β-lactam 類之抗生素亦具抗藥性，包含分子類(如紅黴素)、四環黴素、克林達黴素(clindamycin)、胺基醣苷類(如 gentamicin)、氟化恩琵類(如 ciprofloxacin)、及複方磺胺類，而台灣之 MRSA 抗藥性更高。治療嚴重 MRSA 感染病患的最常用的後線抗生素為萬古黴素(vancomycin)，但近年來日本、美國及其它國家已發現對 vancomycin 有具感受性降低及具抗藥性的菌，台灣亦已有個案報告。

1990 年代時，MRSA 在不同國家醫療機構引起住院病人院內感染之個案逐年增加，近年來許多國家也發現社區感染之個案遽增，且有群突發案例發生，如美式足球隊員、監獄犯人等群體感染之事件。但近年來醫院感染及社區感染個案之區分、定義、及其危險因子(risk factors)，包含病人與醫療機構之接觸史，逐漸模糊，故原 hospital-acquired 及 community-acquired 簡稱 H-MRSA 及 C-MRSA 已被 hospital-onset

及 community-onset 或 hospital-associated 及 community-associated 所取代。H-MRSA 及 C-MRSA 之表現型及基因型有許多相異之處。到目前為止，許多其他國家之 H-MRSA 多具 SCC*mec* types II 或 III，而 C-MRSA 則大多具 SCC*mec* type IV。C-MRSA 對上述之非 β-lactam 類抗生素，尤其是 ciprofloxacin 及 SXT，較無抗藥性。另 C-MRSA 跟 H-MRSA 明顯不同之處是其毒素因子，C-MRSA 大多帶有 PVL(Panton-Valentine leukocidin)毒素。PVL 是一種能破壞白血球之毒素，而被具有此 PVL 毒素 MRSA 感染之病狀，可從局部皮膚感染至嚴重疾病包含壞死性肺炎(necrotizing pneumonia)，甚至死亡。這些不同基因型之 MRSA 對非 β-lactam 抗生素之抗藥性亦明顯不同，故了解基因型的分佈可協助了解抗藥趨勢的改變。

此計畫已建立了 MRSA 分子流行病學研究之國際標準測試平台，可用於跟不同國家地區盛行之 MRSA 比對，以偵測 MRSA 之國際擴散。此計畫比較使用不同表現型方法測試台灣 *E. coli* 及 *K. pneumoniae* 具 ESBL 及 AmpC ß-lactamase 菌之資料，亦將提供給醫院微生物實驗室參考，以增加偵測這些多重抗藥菌之準確度，進而提供醫生更好的用藥選擇參考。此計畫之總目標為對 TSAR I (1998) 至 TSAR VI（2008）中由各 TSAR 醫院之感控小組判斷為院內感染院內感染(Hospital-acquired infection, HAI)菌種，分年度對不同抗藥菌進行抗藥基因及分子流行病學研究。結果顯示抗藥菌之防治與控制，除需強制實施感控措施，以減少多重抗藥菌之擴散，亦需嚴謹監控後線抗生素之使用，以減少抗藥菌之產生而造成抗藥基因在不同菌間之轉移。

此計劃今年調查了第六期 TSAR VI (2008)之以下院感菌：抗甲氧苯色葡萄球菌(MRSA)、對 carbapenem 具抗性院感綠膿桿菌及鮑氏不動桿菌(Carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ,CRPA 及 CRAB)、對廣泛性乙內醯氨感受性降低及具抗藥性的院感大腸桿菌(*Escherichia coli*)及克雷白氏肺炎桿菌(*Klebsiella pneumoniae*)、對萬古黴素具抗藥性之腸球菌(VRE)，進行抗藥基因型及分子流行病學之研究。今年亦開始偵測新興抗藥菌，首先為 *S. aureus* 菌中對萬古黴素感受性降低或具混合抗藥性的 MRSA (Vancomycin reduced susceptible or heterogeneous vancomycin intermediate-resistant *S. aureus*, hVISA)。為了解 MRSA 同基因型菌種所造成的疾病、嚴重性、及治療選擇之差異今年亦設計問卷以便更進一步了解其特性及臨床流行病學，以協助提供治療指引。為能較即時偵測新興抗藥菌之產生，亦於 98 年底起對部份 TSAR 醫院進行指標多重抗藥菌之收集。

此研究計畫為建立抗藥基因庫及抗藥菌分子與臨床流行病學系統之起使，使用不同期間引起院內感染之常見多重抗藥菌種，介此了解這些抗藥菌之抗藥機制及分子流行病學及在不同醫院之分佈情況及追蹤其演變。此研究所調查之菌種及基因序列亦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，做為將來抗藥細菌感染突發調查之比較，並協助抗藥基因型及分子與臨床流行病學資料庫之建立。

(二) 材料與方法 (Materials and Methods)

■ **研究對象：**

此計畫為連續型計畫，計劃於去年七月開始，**今年**為第二年，研究對象主要為第六期 TSAR (2008)之院感多重抗藥菌，包含 68 株抗甲氧苯青黴素金黃色葡萄球菌 (Methicillin resistant *Staphylococcus aureus*，簡稱 MRSA)、對 carbapenem 具抗性之綠膿桿菌(株)及鮑氏不動桿菌(33 株)(Carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*，簡稱 CRPA 及 CRAB)、對廣泛性乙內醯氨感受性降低及具抗藥性的院感大腸桿菌及克雷白氏肺炎桿菌(Extended spectrum β-lactam reduced susceptible and resistant *Escherichia coli* and *Klebsiella pneumoniae*，簡稱 ESβ-NS eco and kpn)、對萬古黴素具抗藥性之腸球菌(vancomycin-resistant enterococci，簡稱 VRE)。另外亦調查了 35 株萬古黴素 MIC 為 2 μg/mL 之 MRSA 是否為感受性降低或具混合抗藥性的菌(Vancomycin reduced susceptible or heterogeneous vancomycin intermediate-resistant *S. aureus*, hVISA)

■ **研究及分析方法：**

實驗方法包含：抗藥測試(Antimicrobial susceptibility test)、不同乙內醯氨酶 (ß-lactamase)表現型測試、多種 ß-lactamase 基因之測試及定序、多位基因序列分析法(Multilocus Sequence Typing, MLST)、抗 methicillin 基因夾 *SCCmec* (Staphylococcus Cassette Chromosome *mec*)型分類、及 Panton-Valentine leukocidin (PVL) toxin gene 測試，並使用脈衝電泳法(Pulsed-Field Gel Electrophoresis，簡稱 PFGE)調查菌種間相關性，並與過去不會年度資料比對。實驗方法簡述如下：

I). 抗藥測試 (Antimicrobial susceptibility test). 抗藥測試除使用美國 Clinical and Laboratory Standards Institute (CLSI)之 microbroth dilution 方法測試菌株對不同抗生素之最小抑制濃度(Minimum inhibitory concentration，簡稱 MIC)來判讀其抗敏性，亦加用 Etest 測試或確認其中部份菌對一些抗生素之抗敏性。

II). 乙內醯氨酶表現型測試(β-lactamase phenotypic detection).

Extended spectrum β-lactam reduced susceptible and resistant *Escherichia coli* and *Klebsiella pneumoniae*(簡稱 ESβ-NS eco and kpn)為大腸桿菌及克雷白氏肺炎桿菌中，如其 ceftazidime, cefotaxime,或 aztreonam 抗生素的 MIC 大於或等於 2 μg/ml，則為疑是 ESBL-producer，故先調查其是否會產生 ESBL 外(ESBL confirmatory test)。這些菌亦做 AmpC 表現型試驗，以檢測菌株是否有 class C β-lactamases 類酵素。

i. ESBL confirmatory test (產生 ESBL 菌之確定測試)[7]：將懷疑是 ESBL 之菌從

培養 18-24 小時的培養皿挑取 3-5 個相同菌落至食鹽水，調整菌液濃度至 0.5 McFarland 標準，將菌落畫至 Mueller-Hinter agar plate 後，同時測定 ceftazidime (CAZ)，cefotaxime (CTX)及這兩種藥物加入 clavulanic acid 的最低抑菌濃度。如加入 clavulanic acid 的 CAZ 或 CTX 之 MIC 比沒加入 clavulanic acid 之 MIC 降低八倍以上，即確認是 ESBL producer。

ii. AmpC 乙內醯氨酶表現型測試(AmpC β-lactamase phenotypic detection)：

此部份使用三個不同的方法[8,13,35]，以比較其準確性及敏感性。

A. 雙紙錠協力測試法 (Double Disk Synergy Test, DDST): 將調至 0.5 McFarland 濃度的菌液。將菌液均勻塗佈在 Mueller-Hinton Agar (MHA)上，乾燥後將含有 300 μg aminophenylboronic acid hemisulfate (APB)及 30 μg ceftazidime(CAZ)紙錠及 30 μg cefotaxime(CTX)紙錠以間距 18 毫釐的距離排成直線貼在 MHA 上。放入 35 C 培養箱中培養 16-18 小時後，觀察 CAZ 及 CTX 紙錠與含有 APB 紙錠相鄰處的形狀，若與 APB 紙錠相鄰處有延長的抑制圈，則為陽性反應，若與 APB 紙錠相鄰處無延長的抑制圈，則為陰性反應。

B. 抗生素誘發測試法(Disk Potentiation Test, DPT): 將調為 0.5 McFarland 濃度的菌液均勻塗佈在 Mueller-Hinton Agar (MHA)上，乾燥後將含有 300 μg APB 的 CAZ 紙錠及未含 APB 的 CAZ 紙錠以間距 30 毫釐的距離排成直線貼在 MHA 上，在 35 度 C 培養 16-18 小時後，比較含有 APB 的 CAZ 紙錠及沒含 30 μg 的 CAZ 紙錠之抑制圈大小，若含有 APB 的 CAZ 紙錠之抑制圈比不含 APB 的 CAZ 紙錠大(含)5 mm 則為陽性反應，反之則為陰性反應。

C. 修改之 Hodge 測試法(Modified Hodge Test)：將調為 0.5 McFarland 濃度的菌液均勻塗佈在 Mueller-Hinton Agar (MHA)上，乾燥後將含有 30 μg 的 cefoxitin 紙錠貼在 MHA 正中央的位置，再以取菌環沾取 2~3 個測試菌株的菌落由紙錠邊緣劃直線至培養皿邊緣。放入 35 度 C 培養 16-18 小時，觀察紙錠旁之生長抑制圈及測試菌株生長線處是否有抑制圈減少或扭曲的現象；若有則為陽性反應，無則為陰性反應。

III). ESβ-NS 大腸桿菌及克雷白氏肺炎桿菌分子流行病學研究。

i. **脈衝電泳法(Pulsed-Field Gel Electrophoresis, PFGE):**以膠體包埋法(SeaKem Gold Agarose, Lonza)抽取菌株 DNA 後，用 *Xba*I (10 Units, New England BioLabs)的限制酵素於 56 度 C 的水浴槽中作用 2 小時，再以初始轉換時間 (initial switch time)2.16 秒及最終轉換時間 (final switch time)54.17 秒，30~600 kbp 的範圍用 CHEF MAPPER 電泳系統 (Bio-Rad)跑電泳 19 小時後將膠體放入 1 μg/ml 的溴化乙苯非啶溶液(ethidium bromide)中染色，再用紫外光檢測產物並存檔分析。

ii. **ESBL and AmpC β-lactamase gene detection：**使用多對引子聚合酶連鎖反應法(Multiplex PCR)參照文獻[1,22-25,28,29,34]，每一反應管中整體積為 50 μl，含有 20 mM Tris-HCl (pH 8.4)、50 mM KCl、0.2 mM dNTP、1.5 mM $MgCl_2$、不同引子之序列(Table 1 and Table 2)及濃度列於下表、1.25 U 的 Taq DNA 聚合酶及 2 μl

的測試菌株 DNA。聚合酶連鎖反應之流程如下：94°C-3 min，25 cycle 之 94°C-30 sec; 64°C-30 sec; 72°C-1 min，及 72°C-7 min。反應做完後以 5 µl 的反應物用 2% 膠體跑電泳，跑完後以 Ethidium bromide 染色，再用 UV 檢測是否有聚合酶反應之產物。

IV). MRSA 抗藥基因及分子流行病學研究

包含 PFGE、*SCCmec* typing、PVL toxin gene 測試，並由 PFGE 結果挑選主要脈衝電泳型別(pulsotype)進行 MLST 之序列型別(Sequence type, ST)。並挑選部份 MRSA 進行 Modified Population Analysis Profile(PAP)測試以找 hVISA (heterogeneous vancomycin intermediate *S. aureus*)簡述簡述如下：

i. **MRSA 脈衝電泳法(Pulsed-Field Gel Electrophoresis, PFGE)**: 實驗根據美國 CDC 建立之標準操作，使用 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 以電泳槽 CHEF-Mapper 跑膠質，電泳後以 0.5 μg/mL ethidium bromide 染色 30 分鐘，清洗後再用紫外光檢測產物並存檔分析。

ii. **MRSA DNA extraction:** 將金黃色葡萄球菌懸浮於 1.5 ml 離心管的培養液中離心 10 分鐘，去除上清液後，以 180 µl 的 lysis buffer 懸浮菌體，並加入 5 µl 濃度為 5 mg/ml 之 lysostaphin，置於 37℃反應 30 分鐘。此後步驟採用 Qiagene DNA extraction kit 之 Gram-positive bacteria 方法(Qiagene DNAeasy Blood and Tissue Kit)。

iii. ***SCCmec* typing:** 使用 multiplex PCR，測試 MRSA 之 *ccr* 及 *mec* 之型。PCR 之步驟基本如下：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，經過 30 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。*SCCmec* 型之判斷是由以下列組合決定。

SCCmec type	*Ccr*	*Mec*
Type I	Type-1 *ccr* (*ccrA1* and *B1*)	Class B *mec*
Type II	Type-2 *ccr* (*ccrA2* and *B2*)	Class A *mec*
Type III	Type-3 *ccr* (*ccrA3* and *B3*)	Class A *mec*
Type IV	Type-2 *ccr* (*ccrA2* and *B2*)	Class B *mec*
Type V	Type-5 *ccr* (*ccrC*)	Class C *mec*

iv. **PVL toxin gene detection:** Primer 及 PCR 參考文獻[21]。

iv. **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 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(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)，進而與世界其他國家之 MRSA 之 ST 做比對，以便追蹤 MRSA 之演變。

v. **Modified Population Analysis Profile(PAP)：**將實驗菌株接種於 5 毫升 Trypticase soy broth 並放置 35℃培養箱中震盪培養，次日取出培養液，將菌液濃度調整至 0.5 McFarland 以作為該實驗菌株之起始濃度(約 $1x10^8$ CFU/ml)，並自起始管吸取 0.1 毫升菌液至 0.9 毫升 TSB 中，依序進行六次序列稀釋，共得 7 管不同濃度(10^8 ~10^2 CFU/ml)之實驗管，分別自各實驗管吸取 50µl 菌液至含有不同 vancomycin 抗生素濃度之 BHIA 上，塗抹均勻後置於 35℃培養箱培養，分別於 24、48 小時計數菌落數。此實驗方法原為 Hiramatsu et al 報告，後經 Wootton et al 改較簡易化。

V). Imipenem resistant *P. aeruginosa* 及 *A. baumannii* 研究：包含 metallo-β-lactamase (MBL)表現型測試(phenotypic detection)、PFGE、及使用 PCR 與 DNA sequencing 偵測不同 carbapenemase 之基因。實驗方法簡述如下：

i. **Metallo-β-lactamase (MBL)表現型測試:** 使用 E test 方法(AB Biodisk)，測試對 imipenem 或 meropenem 不具感受性(intermediate or resistant)之 *P. aeruginosa* 與 *A. baumannii* 菌。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 agar 後，將含 imipenem 及 imipenem + EDTA 的 Etest strip 放上，培養於 35°C 18 小時後，判讀最低抑菌濃度(minimum inhibitory concentration, MIC)。如含 EDTA 的 imipenem 之 MIC 比沒加入 EDTA 的 imipenem 之 MIC 至少降低八倍，即確認是 MBL producing 菌。

ii. **PCR 和 DNA sequencing：**以 PCR 的方法偵測各類 carbapenemase β-lactamases 酵素基因[19,26]。其中 A 類 Carbapenemases 包括：NMC、SME、IMI、KPC、GES，D 類 Oxacillinases 包括：OXA-23、OXA-24、OXA-69、OXA-58、*Shewanella* OXA-55、OXA-48、OXA-50、OXA-60，以及在 *A. baumannii* 之 OXAs 混合 PCR 中包括：OXA-51-like、OXA-23-like、OXA-24-like、OXA-58-like，以上之反應條件為：95℃ 10 分鐘；30 循環（94℃ 30 秒；50℃ 30 秒；72℃ 1 分鐘）；最後 72℃ 10 分鐘。B 類 Metallo-enzymes 包括：IMP-1、IMP-2、VIM-1、VIM-2、SPM-1、GIM-1、SIM-1，反應條件為：94℃ 3 分鐘；35 循環（94℃ 1 分鐘；55℃ 1 分鐘；72℃ 2 分鐘）；最後 72℃ 7 分鐘。PCR 反應產物以 1.5%瓊脂電泳分析，經 EtBr 染色與照相判斷結果。確定分子量之 PCR 反應產物取 5 µl 與純化酵素（EXO-SAP enzyme） 2 µl 經 37℃ 20 分鐘及 80℃ 15 分鐘反應純化後，將其加入引子（primer）混合後進行核酸定序，核酸定序結果以 *BLAST* 程式分析（http://www.ncbi.nlm.nih.gov/*BLAST*）。

iii. **脈衝式電泳法分型（Pulsed field gel electrophoresis, PFGE）**：將調好適當濃度的

菌液與等量 1%瓊脂（SeaKem Gold agarose in TE buffer）及 20 µl 之 20 mg/ml Proteinase K 混合均勻後，注滿於鑄膠模具（plug mold），待其冷卻固化形成膠塊（plug），將膠塊放入分裝有 3 ml 細胞溶解緩衝液（含有 20 mg/ml Proteinase K 20µl）之 50 ml 離心管中，於 56℃水浴槽震盪 2 小時，之後將膠塊放入有孔篩子中，串連後利用循環機器以二次水清洗兩次，再以 1X TE Buffer 清洗兩次，清洗後的膠塊保存於 1X TE Buffer 4℃冰箱中。將要分析的膠塊放入分裝有 200 µl 限制酵素專用緩衝液的 1.5 ml 微量離心管中，置於室溫下震盪 5~10 分鐘，移除緩衝溶液，再加入 200 µl 已含有酵素的限制酵素(restriction enzyme)專用緩衝溶液【*P. aeruginosa*: 30U, *SpeI*, 37℃, overnight；*A. baumannii*: 30U, *ApaI*, 25℃, 4 小時；標準菌株為金黃色葡萄球菌 *S. aureus* NCTC8325, 20U, *SmaI*, 25℃, 4 小時，作用時間結束後，移除酵素專用緩衝溶液，置換成 200 µl 的 0.5X TBE 緩衝液。脈衝式電泳儀器為 Bio-Rad CHEF Mapper XA system，電泳用膠為 1% SeaKem Gold agarose，緩衝溶液為 0.5X TBE，電泳條件為 pulse time: 1 s ~ 30 s, 14℃, 6V/cm, 24 hrs [24]。電泳結束後，以 EtBr 染色再用紫外光檢測產物並存檔分析。

VI). 對萬古黴素具抗藥性之腸球菌(vancomycin-resistant enterococci，簡稱 VRE）

i. **Multiplex PCR 抗藥性分型與菌株鑑定:** 使用 multiplex PCR 測定 VRE 的抗藥性分型基因 *vanA* 及 *vanB*，並同時測試分別對 *E. faecalis* 與 *E. faecium* 具有專一性的 *ddlefa* 與 *ddlefm* 基因。實驗流程如下：PCR 每一反應管中總體積為 25 µl，內含 1X buffer、2.5 nM dNTP、2 mM $MgCl_2$、1 U Taq polymerase、並加入核酸引子(primer)各 15 picomole 與細菌 DNA。PCR 反應條件為： 94℃ 5 分鐘；30 循環（94℃ 1 分鐘；54℃ 1 分鐘；72℃ 1 分鐘）；最後 72℃ 10 分鐘。PCR 反應產物以 1.5%瓊脂電泳分析，經 EtBr 染色與照相判斷結果。

ii. **脈衝式電泳法分型（Pulsed field gel electrophoresis, PFGE）**：調好適當濃度的 150 µl 菌液與等量 1%瓊脂（SeaKem Gold agarose in TE buffer）及 6 µl 之 5 mg/ml lysostaphin 與 6 µl 之 5 U/µl lysostaphin 混合均勻後，注滿於鑄膠模具（plug mold），待其冷卻固化形成膠塊（plug），將膠塊放入分裝有 2 ml 細胞溶解緩衝液（含有 20 µl 之 5 mg/ml lysostaphin 與 20 µl 之 5 U/µl lysostaphin）之 50 ml 離心管中，於 54℃水浴槽震盪 1 小時，之後以 1X TE Buffer 清洗一次後，將膠塊放入含有 20 mg/ml Proteinase K 20µl 的緩衝液中，置於 50℃水浴槽 16-20 小時，接著利用循環機器以二次水和 1X TE Buffer 清洗完成後，將膠塊保存於 1X TE Buffer 4℃冰箱中。將要分析的膠塊以 5µl 限制酵素 *SmaI* 作用完成後，進行脈衝式電泳。脈衝式電泳儀器為 Bio-Rad CHEF Mapper XA system，電泳用膠為 1% SeaKem Gold agarose，緩衝溶液為 0.5X TBE。*E. faecium* 菌株之電泳條件為 AutoAlgorithim : 25~400 kbp, 26 hrs。電泳結束後，以 EtBr 染色再用紫外光檢測產物並存檔分析。

VII). 核酸定序：確認約 DNA 產物後，如需要則純化 PCR 產物進行核酸定序步驟。去氧核醣核酸序列分析 (DNA sequencing): 加入 PCR 反應溶液純化配後，置定序送件溶

液，將有聚合酶產物的反應管中取出 5 µl 的反應物，加入 2 µl 的 ExoSAP-IT 酵素，放入機器以 37^{o}C 20 分鐘接 80^{o}C 15 分鐘步驟來純化產物；純化後的產物加入 7 µl 的無菌水，混合均勻後分成兩組 7 µl 的管子，一組加 forward 引子，一組加 reverse 引子，送至國衛院核心實驗室做定序，定序結果以可組合序列之軟體先將 forward 及 reverse 端組合(如 Chromas、VectorNTI 等)之後，即與 PubMed 上之菌株或相關報告中的菌株之 DNA 及蛋白質序列比對。

VIII) 資料分析：使用世界衛生組織之 Whonet 分析軟體，分析這些細菌對不同種類抗生素之抗藥性，因為 CLSI 於 2010 年將改變 *Enterobacteriaceae* 之部份 β-lactam 之感受性標準，故亦比較此判讀標準改變對 ES 抗敏比率之影響。統計學分析則使用 Epi Info 6.04 軟體(CDC, Atlanta, GA)。

IX). 脈場膠電泳分析(Pulsed field gel electrophoresis, PFGE)分析：以 BioNumerics 電腦軟體，進行分型圖譜 dendrogram 電泳相似性（similarities）比對，主要根據 D 係數（Dice coefficients）計算公式，即兩菌株彼此相對位置相同之帶狀片斷數目乘於 2 再除以二者片斷數目總合，為 D 係數。當這些菌株 D 係數≧0.8 時，亦參照 Tenover et al 之規則[32]。當這些菌株 DNA bands 之差異低於 6 條時，即被認為來自相關菌源即被認為來自相關菌源，稱為 pulsotype，而各 pulsotype 中如有少數 DNA banding 差別，則稱為 subtype (如：A1, A2 或 C1, C2 等)。存取之影像檔使用 BioNumerics 分析軟體分析，並以 Dice (Optimization:1.0%, Tolerance: 1.0%)的條件製作 dendrogram 樹狀圖。菌株間之相似性亦參照 Tenover et al 之規則。

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

- **Methicillin resistant *Staphylococcus aureus* (MRSA)**

A. **Nosocomial MRSA in TSAR VI (2008).**

Among the 798 non-duplicate *S. aureus* collected from 26 hospitals in TSAR VI (2008), 538 were from inpatients including 98 from hospital-acquired infections (HAI). A total of 68 (69.4%) of the nosocomial isolates were methicillin resistant including 43 from sterile body sites (34 from blood). The other 25 isolates were from respiratory (7), urine (4), and wound (14) specimens. Based on pulsed field gel electrophoresis (PFGE) results, these 68 isolates can be grouped into 4 main distinct pulsotypes (clonotypes), with 58.8% (40), 8.9% (6), 8.9% (6), and 14.7% (10) of the isolates belonging to pulsotypes A, B, C, and D, respectively (Figure 1). Isolates within the same main pulsotype (A, B, C or D) are either indistinguishable from each other, closely related or possibly related to each other, and most shared ≥80% similarity in PFGE patterns. These 4 main pulsotypes comprised 91.2% of the nosocomial MRSA in TSAR VI (2008).

A summary of the major phenotypic and genotypic characteristics of these 4 major MRSA clones is listed in Table 2 based on data from nosocomial MRSA combining data from 1998 to 2008. Isolates within the same pulsotype share very similar genetic background in sequence type, SCC*mec* type and toxic profile. Each pulsotype also has distinct non-β-lactam resistance profile, with the predominate pulsotype A being most resistant while pulsotype C was least resistant but carries the virulent PVL toxin genes. Differences in the distribution of these pulsotypes help to explain the changes observed in resistance rates in different years.

B. **Detection of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) in TSAR collection.**

A total of 35 MRSA isolates including 9 from nosocomial infections were selected for hVISA screening. The vancomycin MICs of these isolates were 2 μg/mL, which is the upper limit of the susceptible breakpoint based on CLSI (REF). The modified population analysis profile (PAP) method was used and an isolate is considered a possible hVISA if the ratio of the area under the curve (AUC) of the test organism to that of the positive control stains was >90%. Figure 2 shows a negative (top graph) and a positive (bottom graph) hVISA PAP results. Eight isolates tested positive for hVISA including 3 nosocomial isolates by the modified PAP test. We will perform further characterization on these hVISA isolates next year.

C. Active surveillance for MRSA carriage in ICU patients.

This year we also conducted a survey on patients admitted to ICUs of a regional hospital. Patients were screened at admission to ICU and every 3 days while in ICU. A total of 219 nasal cultures from 84 patients were performed between June and August. MRSA was detected in 45 nasal samples (20.5% of samples) from 19 patients (22.6% of patients), indicating a high rate of MRSA carriage in this high risk group of patients. Questionnaires are being used to identify risk factors and transmission of MRSA among these ICU patients.

D. Discussions on MRSA studies

Nosocomial MRSA in Taiwan belong to 4 major clones, each with distinct clonal lineage and antibiogram. Our previous study had found that the distribution of these 4 clones varied in years and specimen types. Investigation of clinical data on patients from whom MRSA were isolated is currently in progress using a pre-designed questionnaire. This will help us to understand the disease spectrum, risk factors, infection status, and treatment outcomes associated with these 4 major MRSA clones. Clinical investigation of hVISA isolates will also be performed.

■ **Extended-spectrum β-lactam reduced susceptible and resistant (ESβ-NS) *Escherichia coli* and *Klebsiella pneumoniae* from nosocomial infections.**

A.**Detection of β-lactamase genes *bla*$_{\mathbf{ESBL}}$ and *bla*$_{\mathbf{AmpC}}$.**(Table 3)

Among the 60 ESβ-NS *E. coli* isolates, *bla*$_{\mathrm{AmpC}}$ was detected in 54 isolates (51 CMY-type and 4 DHA-type, including 1 isolate having 2 types) (90%), including 19 (31.7%) carrying both *bla*$_{\mathrm{ESBL}}$ and *bla*$_{\mathrm{AmpC}}$. Another 6 isolates were ESBL confirmatory test positive but *bla*$_{\mathrm{ESBL}}$ was detected only in 5 (8.3%, all CTX-M type). There were 3 *bla*$_{\mathrm{AmpC}}$ positive isolates which were also *bla*$_{\mathrm{ESBL}}$ positive by PCR but their ESBL phenotypic confirmatory test results were negative. In the 38 ESβ-NS *K. pneumoniae*, 21 isolates were ESBL-confirmatory test positive but *bla*$_{\mathrm{ESBL}}$ was detected only in 18 isolates (47.4%) (10 CTX-M and 8 SHV type *bla*$_{\mathrm{ESBL}}$), 7 (18.4%) of which also carried *bla*$_{\mathrm{AmpC}}$ (DHA-type). Another 7 isolates (18.4%) carried just *bla*$_{\mathrm{AmpC}}$ (1 CMY-type and 6 DHA-type). Taken together, these data indicated that *blaAmpC* has become the major mechanisms of resistance in ESβ−nonsusceptible isolates, especially in *E. coli.* In addition, false -positive and –negative phenotypic ESBL confirmatory test do occur.

In TSAR VI (2008), a total of 60 (48%) of the 125 nosocomial *E. coli* and 38 (44%) of the 86 *K. pneumoniae* nosocomial isolates were ESβ-NS (ESBL-suspect). The Clinical

and Laboratory Standards Institute (CLSI) had recommended that ESBL confirmatory test should be performed on ESBL-suspects (isolates with aztreonam, ceftazidime or cefotaxime MIC $\geq$ 2 μg/mL) and if the isolate was a confirmed ESBL producer, all cephalosporin drugs should be reported as resistant. However, in 2010, CLSI will be lowering the interpretive breakpoints for several β-lactam drugs including aztreonam, cefotaxime, and ceftazidime so that routine ESBL testing will not be required. Since we had performed ESBL confirmatory test on all ESBL suspects, we compared these nosocomial isolates (Table 4) using the ESBL-producer criteria and 2010 new breakpoints to see the effect of such changes on resistance rates and found them to be quite similar. The change in CLSI breakpoints in 2010 may alleviate some of the phenotypic test problems.

B. Clonal relatedness of ESβ-NS nosocomial *E.coli* and *K. pneumoniae* from 2008.

PFGE was performed on XbaI digested chromosomal DNA of the ESβ-NS *E. coli* and *K. pneumoniae* isolates described above to determine if a particular clone predominated or if there were inter- and intro- hospital clonal spread (Fig 3 & 4). One pair each of *E. coli* and *K. pneumoniae* from one hospital were identical to each other, while several pairs of *K. pneumoniae* isolates from the same hospitals shared high similarity in PFGE patterns, indicating that different strains of these pathogens may evolve as they acquire resistance determinants, but intra-hospital spread also occurred.

■ **Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA)**

Among the 641 *P. aeruginosa* isolates collected in TSAR VI (2008), 70 were from nosocomial infections. The rates of carbapenem resistance *P. aeruginosa* have remained similar in recent years, at 7%～9% overall and 10%～11% in nosocomial isolates, for the last 3 rounds of TSAR. Dendrogram of PFGE on the 34 nosocomial CRPA from 2004, 2006, and 2008 revealed distinct patterns except a couple pairs of isolates from the same hospital having identical patterns (Fig 5). Five of these 34 CRPA were positive for $bla_{\text{VIM-2 or -3}}$ including 2 from 2008. Thus the prevalence of metallo-β-lactamase among CRPA was 14.7% (5/34). The mechanisms of carbapenem resistance in the other nosocomial CRPA remains unknown at the present time.

■ **Carbapenem-resistant *Acinetobacter baumannii***

A. **Antimicrobial resistance and clonal relatedness of CRAB.**

A major infection control challenge facing the medical community in Taiwan is carbapenem resistant *A. baumannii* (CRAB). CRAB has escalated to an alarming high rate in Taiwan hospitals. Among *A. baumannii* from ICU, CRAB was 19% (20/105) in 2004, 44.7% (46/103) in 2006, then sharply increased to 68.8% (86/125) in 2008. In

2002, 12.2% (5/14) of the nosocomial *A. baumannii* were CRAB. That rate increased to 33.8% (23/68) in 2006, and reached 50.8% (33/65) in 2008. The majority (75%) of CRAB were from elderly patients (>70 yo), indicating that CRAB may be concentrated in specific patient populations. Most CRAB are also resistant to other antimicrobials commonly used to treat infections caused by this pathogen, including amikacin (91%), ampicillin/sulbactam (78%), cefepime (76%), ceftazidime (97%), levofloxacin (86%) and piperacillin/tazobactam (98%) so treatment options for CRAB are quite limited. PFGE of the nosocomial CRAB from 2008 revealed a predominant cluster of isolates from different hospitals having >80% similarity (Figure 6). Several isolates from within the same hospital also showed high similarity. Further investigations are needed to determine the clonal relationship of this cluster and see if there is a clonal spread.

B. ***Acinetobacter baumannii* genomic species identification.**

Because conventional biochemical tests do not differentiate the 4 genomic species in the *A. baumannii* complex (*A. clacoaceticus-A. baumannii* complex, *A. calcoaceticus*, *A. baumannii*, and genomic species 3, and 13TU), and because *A. baumannii* and *Acinetobacter* species 13TU are more commonly associated with multidrug resistance and nosocomial infections, we used PCR to differentiate these 2 genospecies and confirmed that all 2008 nosocomial CRAB were *A. baumannii*.

C. **Detection of carbapenemase genes and their association with IS*Aba1*.**

In addition to using PCR to look for different carbapenemases listed in Table 1, we also investigated the association of IS*Aba*1 with the $bla_{\text{oxa.}}$carbapenemases OXA-23 and OXA-51. Carbapenem resistance relative to presence and location of IS*Aba1* and oxacillinase gene is shown in Table 3. $bla_{\text{oxa-51}}$ is considered intrinsic to *A. baumannii* thus were detected in both carbapenem -susceptible and -resistant *A. baumannii* isolates. In carbapenem-resistant *A. baumannii*, IS*Aba1* was closely associated with the presence of $bla_{\text{oxa-51.}}$ However, if $bla_{\text{oxa-23}}$ was detected in addition to $bla_{\text{oxa-51}}$, the IS*Aba1* was associated with $bla_{\text{oxa-23}}$ instead. We have also performed PCR using IS*Aba1* forward primer and $bla_{\text{oxa-23}}$ reverse primer, and IS*Aba1* forward primer and $bla_{\text{oxa-51}}$ reverse primer. Sequencing results revealed that IS*Aba1* was present immediately upstream of the bla_{oxa} gene in CRAB. It has been reported that these IS*Aba1* provides strong promoter sequences to enhance the expression of the bla_{oxa} ß-lactamase genes. Although both IS*Aba1* and $bla_{\text{oxa-51}}$ were detected in isolates susceptible to carbapenem, there were no products from the IS*Aba1*-$bla_{\text{oxa-51}}$ PCR, indicating that IS*Aba1* are not immediately upstream of $bla_{\text{oxa-51}}$ in the carbapenem-susceptible isolates (Table 5)

D. **Outbreak investigation assistance**

This year we also assisted two hospitals in investigation of carbapenem-resistant *Acinetobacter baumannii* outbreaks by performing PFGE. One was an investigation of a burn unit involving 6 patients. PFGE of isolates from patients and environmental samples revealed identical pattern of CRAB. The data helped the hospital to implement stringent infection control measures and interventions policies to prevent the further spread of this multidrug resistant pathogen.

■ **Vancomycin resistant enterococci (VRE)**

The prevalence of vancomycin resistant *Enterococcus faecalis* (VREfa) has remained low (<1.0%) in Taiwan, and no VREfa was found from ICU and HAI *E. faecalis* isolates in TSAR VI (2008). In contrast, there was a significant increase ($p < 0.05$) in vancomycin resistant *E. faecium* in 2008, from 4.2% in 2004 (3/71) and 11.7% (9/77) in 2006, to 25.0% (29/116 isolates) in 2008. The 29 vancomycin-resistant *E. faecium* (VREfm) in 2008 were from 11 hospitals but 16 isolates were from 2 hospitals (9 and 7 isolates each). Among the 26 isolates from inpatients, 5 were determined to be HAI by the hospitals but another 16 were from patients hospitalized for more than 2 days (3 to 58 days), thus most of these VREfm could be HAI related. Since this was a significant increase in VRE, we performed genotypic studies on all 29 VREfm by PFGE and determined their vancomycin resistance genes (*van*A and *van*B) (Figure 7). Concordant phenotype and resistance genotype was found, with v*an*A isolates being also teicoplanin-resistant and *van*B isolates being teicoplanin-susceptible. Most of the VREfm had distinct PFGE patterns from each other. However, 3 pairs of isolates from 2 hospitals shared indistinguishable PFGE patterns. Although these 3 pairs were distinct from each pair, isolates within the same pair were from the same hospitals. These results indicated that the increased prevalence of VREfm in Taiwan hospitals in 2008 was in part due to intra-hospital spread. However, VREfm also emerged in several other hospitals. The reason for the increase in VRE in certain hospitals warrants further investigation.

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

MRSA continues to be prevalent (around 70% in nosocomial *S. aureus*) in Taiwan hospitals and 4 clones of MRSA are endemic. Clinical investigation is underway to study if the clinical manifestations differ in these 4 clones since they have distinct genetic lineage and antibiogram. MRSA with heterogeneous vancomycin-intermediate resistance is an emerging resistance to be on the lookout for in Taiwan. Resistance to extended spectrum ß-lactams (ESß) in *E. coli* and *K. pneumoniae* has reached near 50% in HAI isolates. Resistance in ESß *E. coli* is now mostly due to AmpC ß-lactamase but isolates co-carrying ESBL and AmpC ß-lactamases are also increasing. ESBL ß-lactamase is still the major mechanism of resistance to ESß in *K. pneumoniae* but AmpC ß-lactamases is also prevalent. Significant increase in VRE in Taiwan hospitals was noted in 2008 and careful monitoring of this emerging resistance is needed to prevent their further spread. There is some evidence of intra-hospital spread of some of these resistant bacteria. This year we also assisted in investigation of 2 carbapenem-resistant *A. baumannii* outbreaks to help the enforcement of infection control interventions. In addition, because TSAR is conducted every 2 years, we have started a collection of selected resistant bacteria from 6 TSAR hospitals for more timely detection of emerging resistant bacteria. Testing of these isolates will be started early next year. In conclusion, because decrease in selective pressure can prevent polyclonal expansion of resistance determinants and enforcement of infection control measures can prevent the further spread of resistant clones, strategies for the control of rising antimicrobial resistance problem should include antibiotic stewardship and enforcement of infection control policy.

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(七) 圖表 Tables and Figures.

Table 1. Primers used for detection of carbapenemase (REF)

Enzyme family	Primer[b]	Primer sequence (5'-3')	Target
Class A carbapenemases			
NMC	NMC1*	GCATTGATATACCTTTAGCAGAGA	NMC-R
	NMC4	CGGTGATAAAATCACACTGAGCATA	
SME	IRS-5	AGATAGTAAATTTTATAG	SME-1
	IRS-6	CTCTAACGCTAATAG	
IMI	IMI-A	ATAGCCATCCTTGTTTAGCTC	imiA
	IMI-B	TCTGCGATTACTTTATCCTC	
KPC	KPC forward	ATGTCACTGTATCGCCGTCT	*bla* KPC-1
	KPC reverse	TTTTCAGAGCCTTACTGCCC	
GES	GES-C	GTTTTGCAATGTGCTCAACG	*bla* GES-2
	GES-D	TGCCATAGCAATAGGCGTAG	
Class B metalloenzymes			
IMP-1	Forward	TGAGCAAGTTATCTGTATTC	*bla* IMP-1
	Reverse	TTAGTTGCTTGGTTTTGATG	
IMP-2	Forward	GGCAGTCGCCCTAAAACAAA	
	Reverse	TAGTTACTTGGCTGTGATGG	
VIM-1	Forward	TTATGGAGCAGCAACCGATGT	
	Reverse	CAAAAGTCCCGCTCCAACGA	
VIM-2	Forward	AAAGTTATGCCGCACTCACC	*bla* VIM-2
	Reverse	TGCAACTTCATGTTATGCCG	
SPM-1	SPM-1F	CCTACAATCTAACGGCGACC	spm-1
	SPM-1R	TCGCCGTGTCCAGGTATAAC	
GIM-1	GIM-1F	AGAACCTTGACCGAACGCAG	*bla* GIM-1
	GIM-1R	ACTCATGACTCCTCACGAGG	
SIM-1	SIM1-F	TACAAGGGATTCGGCATCG	*bla* SIM-1
	SIM1-R	TAATGGCCTGTTCCCATGTG	
Class D oxacillinases			
Subgroup 1 (OXA-23)	P5	AAGCATGATGAGCGCAAAG	*bla* OXA-23
	P6	AAAAGGCCCATTTATCTCAAA	
Subgroup 2 (OXA-24)	Forward	GTACTAATCAAAGTTGTGAA	*bla* OXA-24
	Reverse	TTCCCCTAACATGAATTTGT	
Subgroup 3 (OXA-69)	OXA-69A	CTAATAATTGATCTACTCAAG	*bla* OXA-69
	OXA-69B	CCAGTGGATGGATGGATAGATTATC	
Subgroup 4 (OXA-58)	Pre-OXA-	TTATCAAAATCCAATCGGC	*bla* OXA-58
	PreOXA-58B	TAACCTCAAACTTCTAATTC	
Subgroup 5	OXA-55/1	CATCTACCTTTAAAATTCCC	*bla* OXA-55
	OXA-55/2	AGCTGTTCCTGCTTGAGCAC	
Subgroup 6 (OXA-48)	OXA-48A	TTGGTGGCATCGATTATCGG	*bla* OXA-48
	OXA-48B	GAGCACTTCTTTTGTGATGGC	
Subgroup 7 (OXA-50)	S	AATCCGGCGCTCATCCATC	PAO1
	AS	GGTCGGCGACTGAGGCGG	
Subgroup 8 (OXA-60)	OXA-60 A	AAAGGAGTTGTCTCATGCTGTCTCG	*bla* OXA-60
	OXA-60 B	AACCTACAGGCGCGCGTCTCACGGTG	
ISAba1	ISAba1F	CGCGAATGCAGAAGTTG	*ISAba1*
	ISAba1R	CGACGAATACTATGACAC	

Table 2. Phenotypic and genotypic characteristics of major clones of 345 nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) in Taiwan hospitals (TSAR I to VI combined) [a]

Pulsotype	N (% of total)	Genotypic characteristics (% in pulsotype)			% of isolates resistant to:				
		MLST[b]	SCC*mec* type	*pvl*	CIP	ERY	GEN	SXT	TET
A	249 (72.2)	ST239/241 (27/30)	III (97.5)	0	99.6	100	99.6	97.6	98.8
B	30 (8.7)	ST59 (10/11)	IV (100)	0	0	100	70.0	0	63.3
C	16 (4.6)	ST59 (2/2)	V (100)	100	0	100	12.5	0	50
D	35 (10.1)	ST5 (11/14)	II (100)	0	100	100	97.1	0	2.9

[a] Tested on all 345 isolates except MLST (see below). MLST, multilocus sequence typing, SCC*mec*, Staphylococcal Cassette Chromosome *mec*; *pvl*, genes encoding the Panton-Valentine leukocidin toxin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

[b] MLST was performed on selected representative isolates from each pulsotype. Data shown in parenthesis indicate number of isolates with the sequence type (ST)/number of isolates tested.

Table 3. ESBL and AmpC ß-lactamase phenotypic and genotypic test results on TSAR VI (2008) *Escherichia coli* and *Klebsiella pneumoniae* nosocomial isolates

Species, No. of isolates	Group	Phenotypic tests			Genotypic tests			
		ESBL	AmpC	No.	bla_{ESBL} detected		bla_{AmpC} detected	
					CTX	SHV	CIT	DHA
Escherichia coli HAI isolates: 125, ESBL suspect: 60	A	+	+	16	16	0	15*	2*
	B	+	-	6	5	0	0	0
	C	-	+	38	2	1	36	2
	D	-	-	0	0	0	0	0
Klebsiella pneumoniae HAI isolates: 86, ESBL suspect: 38	A	+	+	7	5	2	0	7
	B	+	-	21	10	8	0	0
	C	-	+	7	0	0	1	6
	D	-	-	3	0	0	0	0

*One isolate had two types of bla_{AmpC}.

Table 4. Effects of changes in CLSI interpretive criteria on rates of resistance in nosocomial *E. coli* (A) and *K. pneumoniae* (B) from 2006 and 2008*.

A. *Escherichia coli*

Antibiotic name	2006 (N=66)						2008 (N=125)					
	2009 CLSI			2010 CLSI			2009 CLSI			2010 CLSI		
	%R	%I	%S	%R	%I	%S	%R	%I	%S	%R	%I	%S
Amikacin	6.1	0	93.9				5.6	0.8	93.6			
Amoxicillin/Clavulanic acid	39.4	15.2	45.5				47.2	13.6	39.2			
Ampicillin	84.8	0	15.2				89.6	0	10.4			
Aztreonam	33.3	1.5	65.2	43.9	0	56.1	32	0.8	67.2	46.4	0	53.6
Cefazolin	48.5	3	48.5	74.2	0	25.8	52	4	44	77.6	0	22.4
Cefepime	33.3	0	66.7	43.9	0	56.1	32.8	0	67.2	46.4	0	53.6
Cefotaxime	33.3	0	66.7	43.9	0	56.1	32	4.8	63.2	46.4	0.8	52.8
Cefoxitin	33.3	7.6	59.1				45.6	1.6	52.8			
Ceftazidime	33.3	9.1	57.6	43.9	0	56.1	32	11.2	56.8	46.4	0.8	52.8
Cefuroxime	45.5	3	51.5	47	1.5	51.5	47.2	5.6	47.2	48	4	48
Ciprofloxacin	43.9	0	56.1				43.2	0.8	56			
Gentamicin	45.5	1.5	53				43.2	1.6	55.2			
Imipenem	0	0	100				0.8	0	99.2			
Piperacillin	77.3	7.6	15.2				81.6	8	10.4			
Trimethoprim/Sulfamethoxazole	60.6	0	39.4				60	0	40			

B. *Klebsiella pneumoniae*

Antibiotic name	2006 (N=65)						2008 (N=86)					
	CLSI 2009			CLSI 2010			CLSI 2009			CLSI 2010		
	%R	%I	%S	%R	%I	%S	%R	%I	%S	%R	%I	%S
Amikacin	26.2	0	73.8				16.3	0	83.7			
Amoxicillin/Clavulanic acid	40	10.8	49.2				34.9	16.3	48.8			
Ampicillin	92.3	6.2	1.5				93	4.7	2.3			
Aztreonam	46.2	1.5	52.3	49.2	0	50.8	37.2	1.2	61.6	43	0	57
Cefazolin	52.3	4.6	43.1	58.5	0	41.5	45.3	2.3	52.3	53.5	0	46.5
Cefepime	46.2	0	53.8	49.2	0	50.8	37.2	0	62.8	43	0	57
Cefotaxime	46.2	0	53.8	49.2	0	50.8	37.2	0	62.8	43	0	57
Cefoxitin	32.3	9.2	58.5				26.7	9.3	64			
Ceftazidime	46.2	3.1	50.8	49.2	0	50.8	37.2	3.5	59.3	43	1.2	55.8
Cefuroxime	50.8	1.5	47.7	50.8	1.5	47.7	44.2	2.3	53.5	44.2	2.3	53.5
Ciprofloxacin	44.6	0	55.4				36	1.2	62.8			
Gentamicin	40	7.7	52.3				36	7	57			
Imipenem	0	0	100				0	1.2	98.8			
Piperacillin	58.5	0	41.5				51.2	4.7	44.2			
Trimethoprim/Sulfamethoxazole	53.8	0	46.2				52.3	0	47.7			

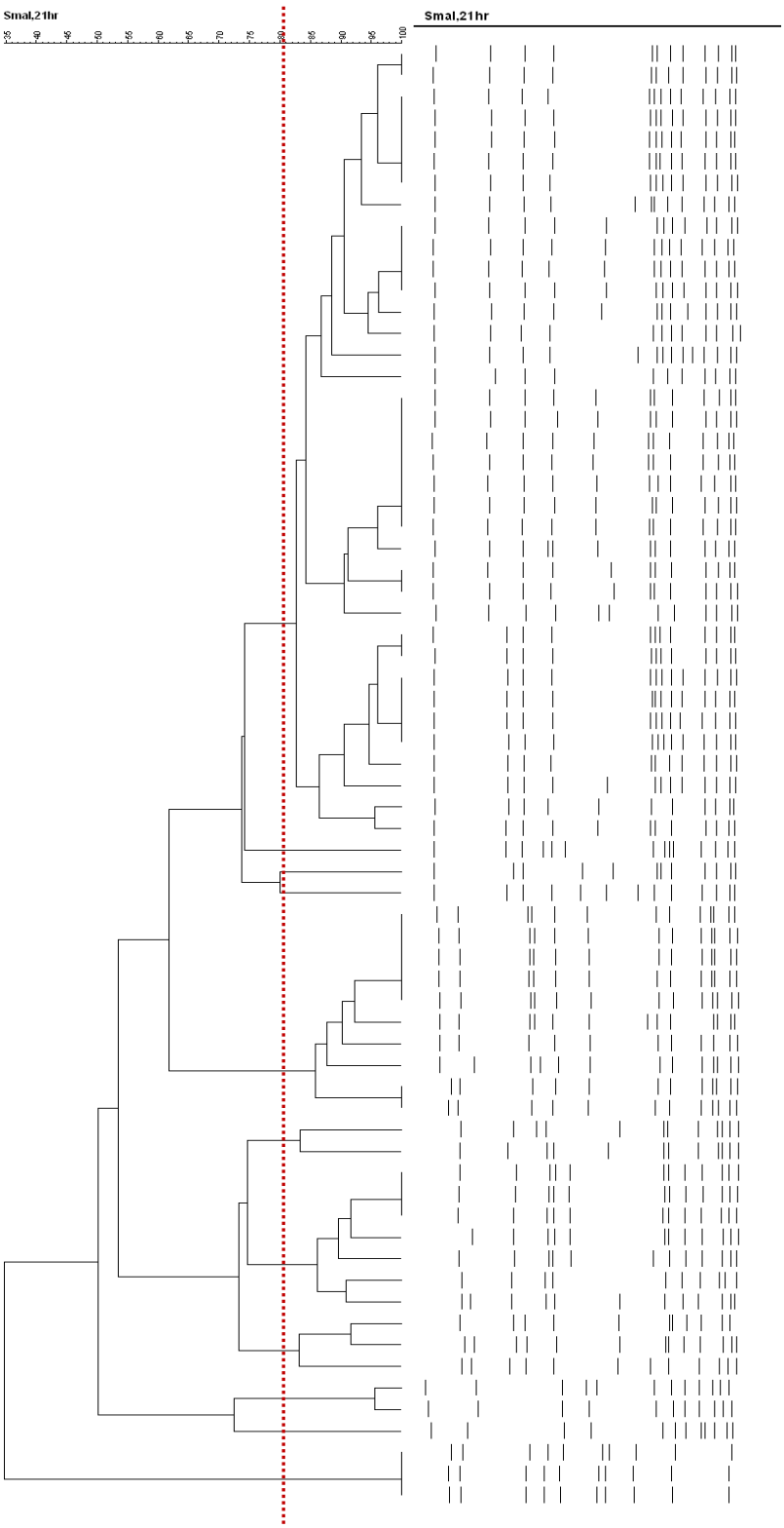
*CLSI, Clinical and Laboratory Standards Instititutes

Table 5. Carbapenem resistance relative to presence and location of IS*Aba1* and *bla*$_{OXA}$ gene

Isolate	MIC (μg/ml) IPM	IS*Aba1*	OXA-gene group	ISAba-IF/OXA23R	ISABA-1F/OXA51R
2008CSM038	0.25	Pos	51	Neg	Neg
2008GTC082	0.5	Pos	51	Neg	Neg
2008MNC165	0.5	Pos	51	Neg	Neg
19606 aba	0.5	Neg	51	Neg	Neg
2008CSM210	6	Pos	51	Neg	**Pos***
2008NCK081	12	Pos*	51*	Neg	**Pos***
2008CSM151	16	Pos	23*,51*	**Pos**	Neg
2008MSH141	32	Pos	23*,51*	**Pos**	Neg
2008SLC053	32	Pos	23*,51	**Pos**	Neg
2008CGK102	>32	Pos	24*,51*	Neg	Neg
2008CSM207	>32	Pos	23*,51	**Pos**	Neg
2008CSM242	>32	Pos	23*,51	**Pos**	Neg
2008SCM152	>32	Pos	23*,51*	**Pos**	Neg
2008SCM156	>32	Pos	23*,51	**Pos**	Neg
2008SCM167	>32	Pos	23*,51	**Pos**	Neg
2008SCM202	>32	Pos	23*,51	**Pos**	Neg
2008SCM230	>32	Pos	23*,51	**Pos**	Neg
2008SCM234	>32	Pos	23*,51	**Pos**	Neg
2008SCM413	>32	Pos	23*,51	**Pos**	Neg
2008SCM414	>32	Pos*	23*,51	**Pos***	**Pos***
2008SLC111	>32	Pos	23*,51	**Pos**	Neg
2008SLC120	>32	Pos	23*,51	**Pos**	Neg
2008SLC132	>32	Pos	23*,51	**Pos**	Neg
2008SLC154	>32	Pos*	51	Neg	**Pos***
2008SLC168	>32	Pos*	23,51	**Pos***	Neg
2008SLC174	>32	Pos	23*,51	**Pos**	Neg
2008SLC222	>32	Pos	23*,51	**Pos**	Neg
2008VGC148	>32	Pos	23*,51*	**Pos**	Neg
2008VGK163	>32	Pos	24*,51*	Neg	Neg
2008VGK171	>32	Pos	24*,51	Neg	Neg
2008YTM172	>32	Pos	51*	Neg	**Pos***
2008ZCC078	>32	Pos	23*,51*	**Pos**	Neg
2008ZCC152	>32	Pos	23*,51	**Pos**	Neg

*PCR product sequenced; 19606 aba is a QC strain.

Figure 1. Dendrogram of 68 nosocomial MRSA from different hospitals in TSAR VI (2008)



MIRL No	Pulsotype	Region	SOURCE TYPE
2008TZH226	A26	N	Blood
2008TZH100	A26	N	Blood
2008NCK153	A11	S	Wound
2008SCM088	A11	M	Wound
2008SCM140	A11	M	Others - SBS
2008SLC126	A11	M	Wound
2008ZGP056	A11	E	Blood
2008KSM064	A cluster	S	Wound
2008CHC225	A7	M	Blood
2008JYC176	A7	S	Resp.
2008VGK116	A7	S	Resp.
2008ZCC122	A7	M	Wound
2008VGK123	A7	S	Others - SBS
2008ZGP135	A49	E	Blood
2008ZGP150	A53	E	Blood
2008TZH211	A cluster	N	Blood
2008CGK051	A40	S	Blood
2008CSM446	A40	M	Blood
2008GTC059	A40	M	Urine
2008VGK157	A40	S	Blood
2008VGK184	A40	S	Others - SBS
2008SLC230	A40	M	Blood
2008THP072	A40	N	Wound
2008NCK229	A cluster	S	Blood
2008SCM439	A41	M	Blood
2008SLC059	A41	M	Blood
2008CGK201	A38	S	Others - SBS
2008SLC150	A9	M	Blood
2008SLC225	A9	M	Wound
2008SLC128	A12	M	Others - SBS
2008SLC180	A12	M	Blood
2008TZH067	A12	N	Resp.
2008VGC166	A12	M	Others - SBS
2008YTM241	A27	N	Blood
2008MNC178	A1	E	Wound
2008LSM070	A52	N	Wound
2008SCM151	A51	M	Resp.
2008NCK092	A cluster	S	Blood
2008SLC160	A cluster	M	Blood
2008VGK097	AJ cluster	S	Urine
2008GTC089	D4	M	Urine
2008KSM166	D4	S	Resp.
2008LSM215	D4	N	Blood
2008MSH129	D4	N	Blood
2008VGC084	D4	M	Wound
2008SCM451	D13	M	Blood
2008THP056	D8	N	Blood
2008ZCC212	D cluster	M	Blood
2008LSM246	D14	N	Blood
2008LSM098	D14	N	Others - SBS
2008SLC088	B cluster	M	Resp.
2008SLC145	B26	M	Blood
2008SCM076	C15	M	Others - SBS
2008SLC067	C15	M	Blood
2008SLC186	C15	M	Wound
2008SLC137	C2	M	Wound
2008SLC246	C10	M	Blood
2008JYC149	C cluster	S	Others - SBS
2008VGK053	B cluster	S	Blood
2008SLC171	B cluster	M	Blood
2008ZGP117	B9	E	Blood
2008HLH191	B cluster	S	Wound
2008GTC103	I cluster	M	Urine
2008SLC077	I1	M	Blood
2008TZH212	I cluster	N	Blood
2008CGK070	E1	S	Wound
2008HLH244-1	E1	S	Blood
2008KSM100	E1	S	Resp.

Figure 2. Population analysis profile (PAP) of two MRSA isolates.

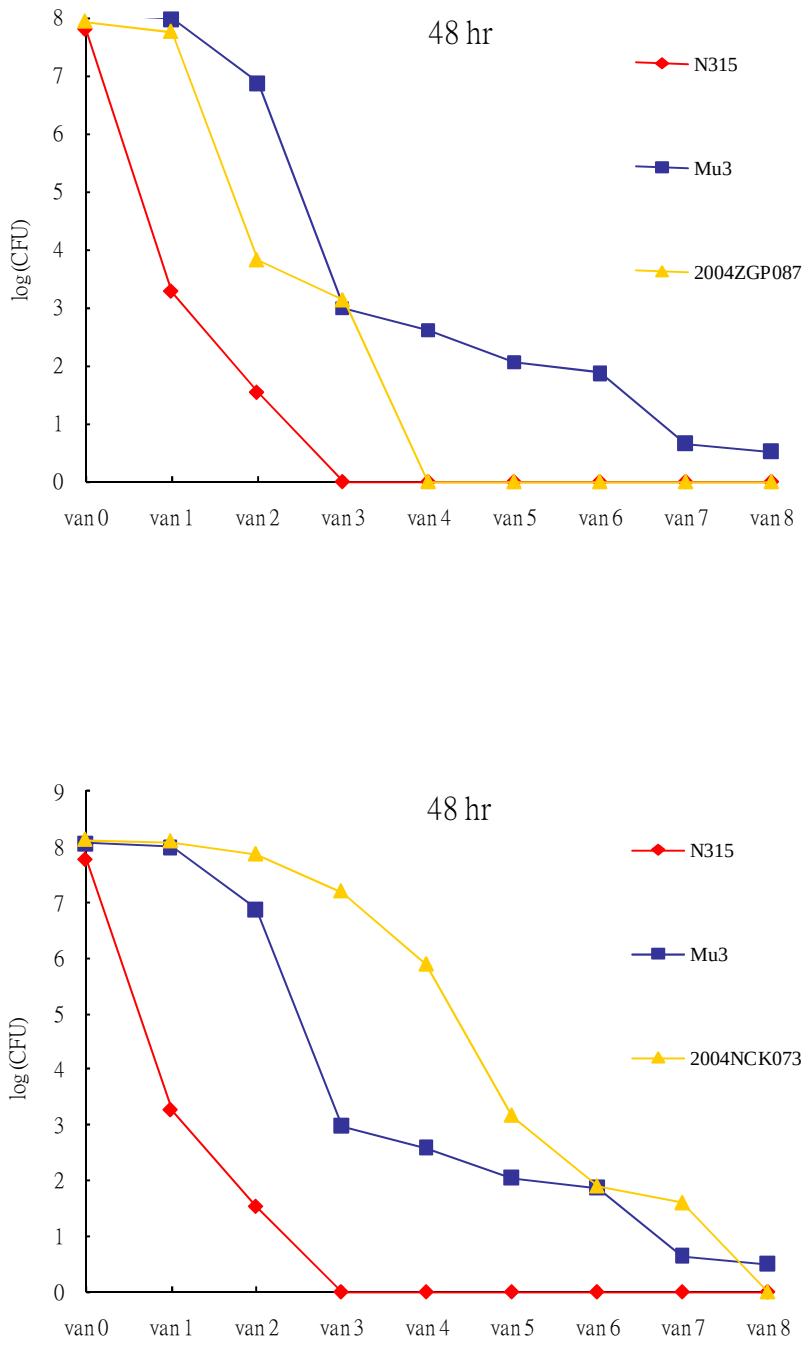
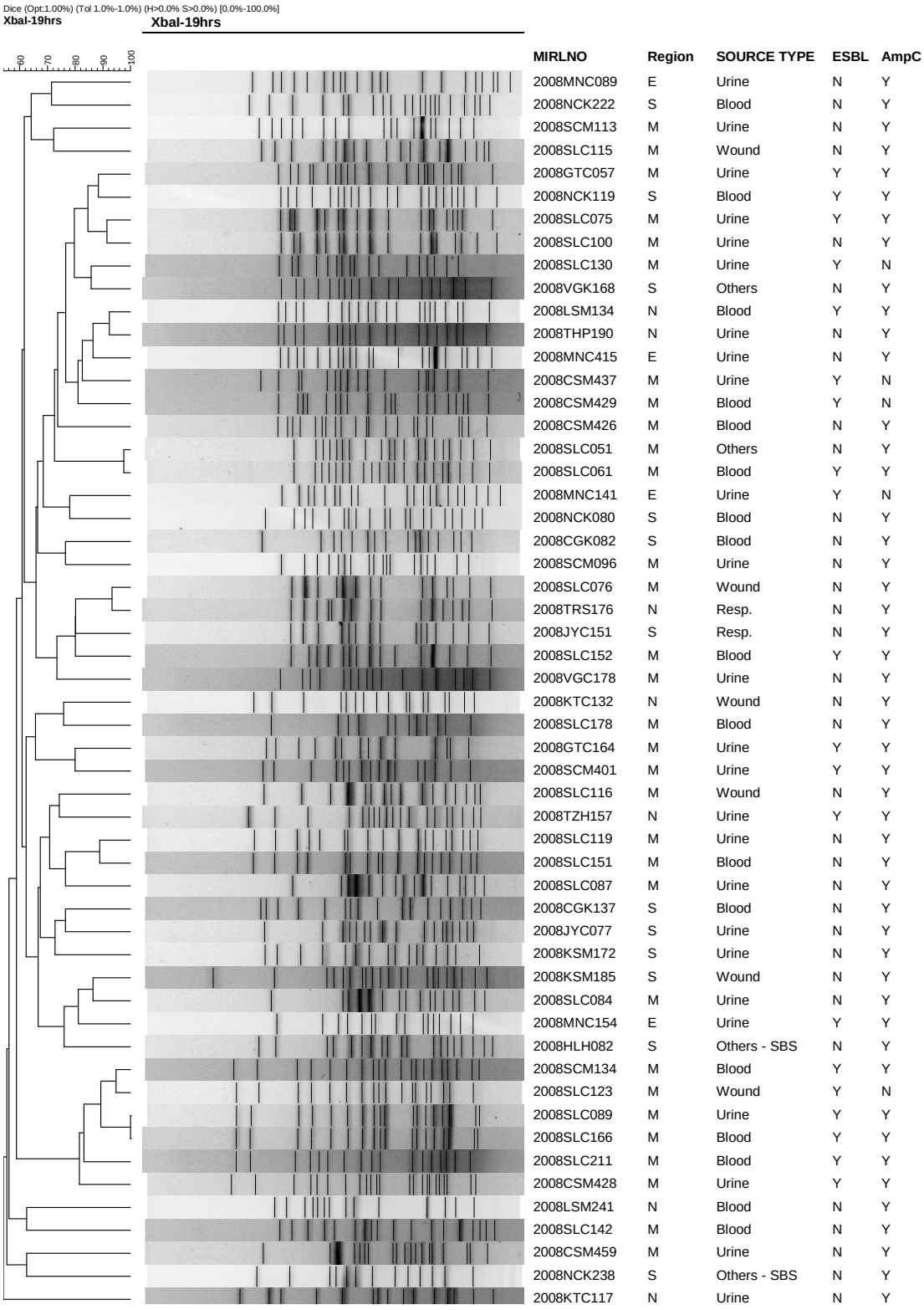


Figure 2 legend. N315, negative control; Mu3, hVISA. Top graph showing test isolate negative for hVISA. Bottom graph showing test isolate positive for hVISA.

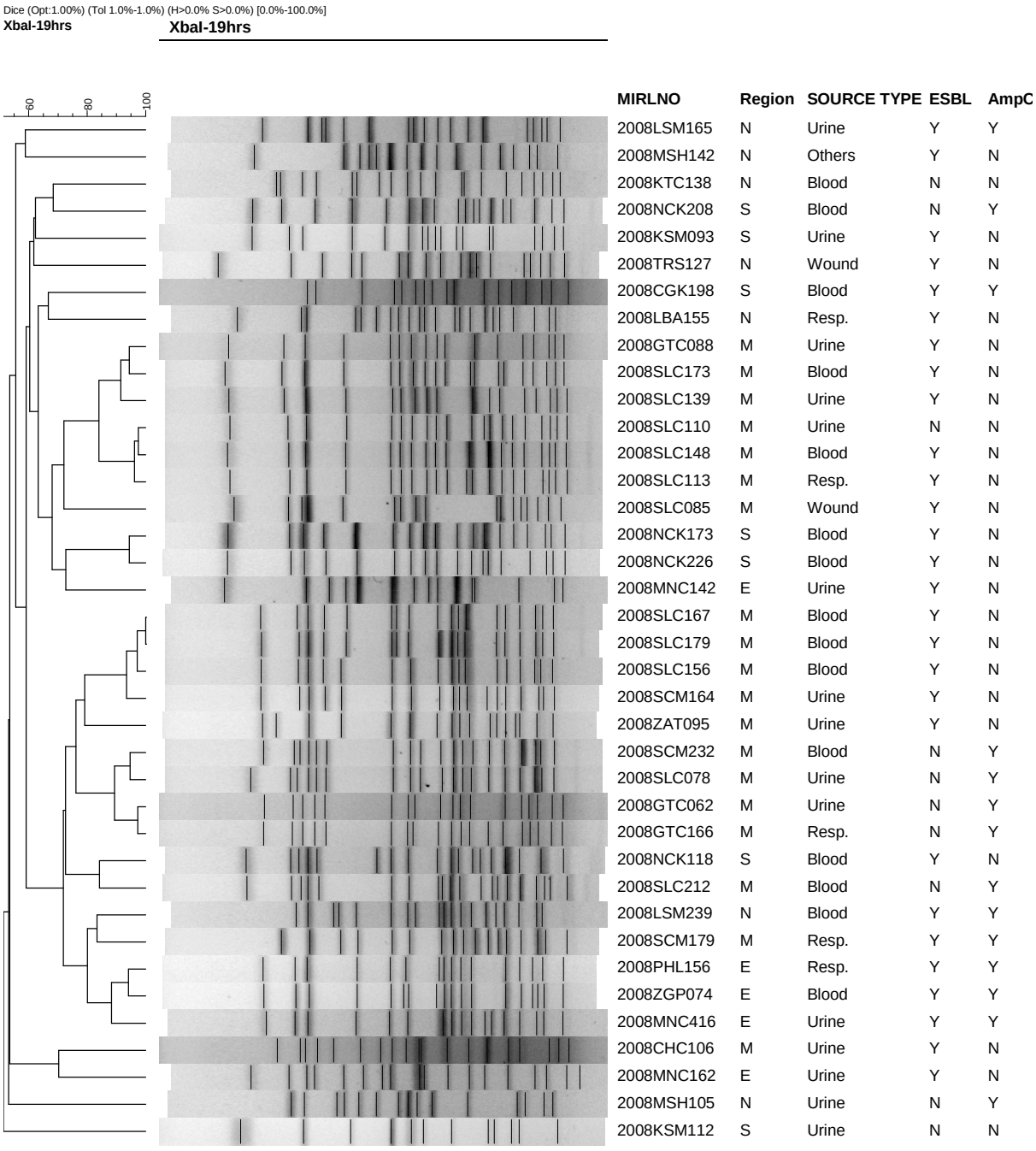
Figure 3. Dendrogram, source, and ß-lactamase genes of 54 nosocomial extended spectrum ß-lactam reduced susceptible and resistant *E. coli* in TSAR VI (2008) (6 isolates could not be typed by PFGE)



MIRLNO	Region	SOURCE TYPE	ESBL	AmpC
2008MNC089	E	Urine	N	Y
2008NCK222	S	Blood	N	Y
2008SCM113	M	Urine	N	Y
2008SLC115	M	Wound	N	Y
2008GTC057	M	Urine	Y	Y
2008NCK119	S	Blood	Y	Y
2008SLC075	M	Urine	Y	Y
2008SLC100	M	Urine	N	Y
2008SLC130	M	Urine	Y	N
2008VGK168	S	Others	N	Y
2008LSM134	N	Blood	Y	Y
2008THP190	N	Urine	N	Y
2008MNC415	E	Urine	N	Y
2008CSM437	M	Urine	Y	N
2008CSM429	M	Blood	Y	N
2008CSM426	M	Blood	N	Y
2008SLC051	M	Others	N	Y
2008SLC061	M	Blood	Y	Y
2008MNC141	E	Urine	Y	N
2008NCK080	S	Blood	N	Y
2008CGK082	S	Blood	N	Y
2008SCM096	M	Urine	N	Y
2008SLC076	M	Wound	N	Y
2008TRS176	N	Resp.	N	Y
2008JYC151	S	Resp.	N	Y
2008SLC152	M	Blood	Y	Y
2008VGC178	M	Urine	N	Y
2008KTC132	N	Wound	N	Y
2008SLC178	M	Blood	N	Y
2008GTC164	M	Urine	Y	Y
2008SCM401	M	Urine	Y	Y
2008SLC116	M	Wound	N	Y
2008TZH157	N	Urine	Y	Y
2008SLC119	M	Urine	N	Y
2008SLC151	M	Blood	N	Y
2008SLC087	M	Urine	N	Y
2008CGK137	S	Blood	N	Y
2008JYC077	S	Urine	N	Y
2008KSM172	S	Urine	N	Y
2008KSM185	S	Wound	N	Y
2008SLC084	M	Urine	N	Y
2008MNC154	E	Urine	Y	Y
2008HLH082	S	Others - SBS	N	Y
2008SCM134	M	Blood	Y	Y
2008SLC123	M	Wound	Y	N
2008SLC089	M	Urine	Y	Y
2008SLC166	M	Blood	Y	Y
2008SLC211	M	Blood	Y	Y
2008CSM428	M	Urine	Y	Y
2008LSM241	N	Blood	N	Y
2008SLC142	M	Blood	N	Y
2008CSM459	M	Urine	N	Y
2008NCK238	S	Others - SBS	N	Y
2008KTC117	N	Urine	N	Y

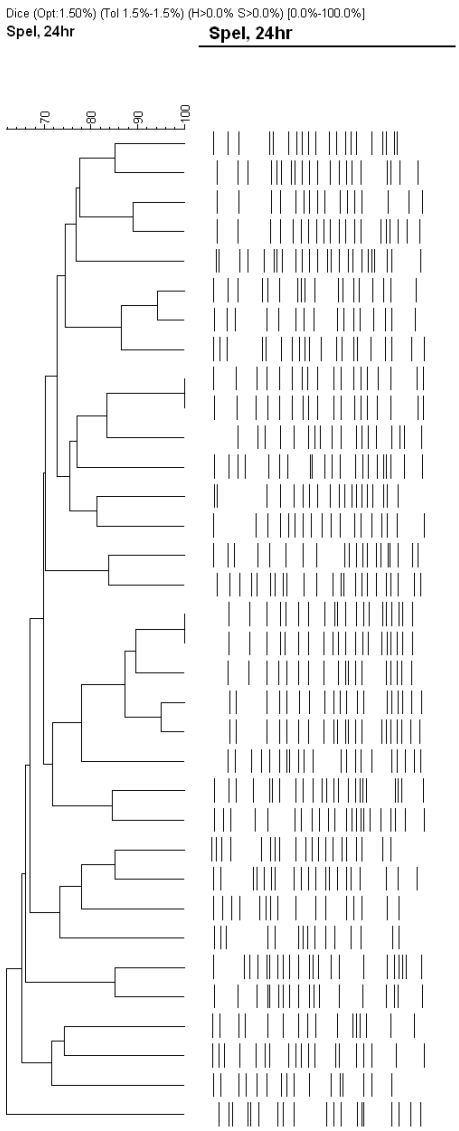
Figure 3 legend. ESBL, bla_{ESBL} ; AmpC, bla_{AmpC}

Figure 4. Dendrogram and ß-lactamase of 38 nosocomial extended spectrum ß-lactam reduced susceptible and resistant *Klebsiella pneumoniae* in TSAR VI (2008)



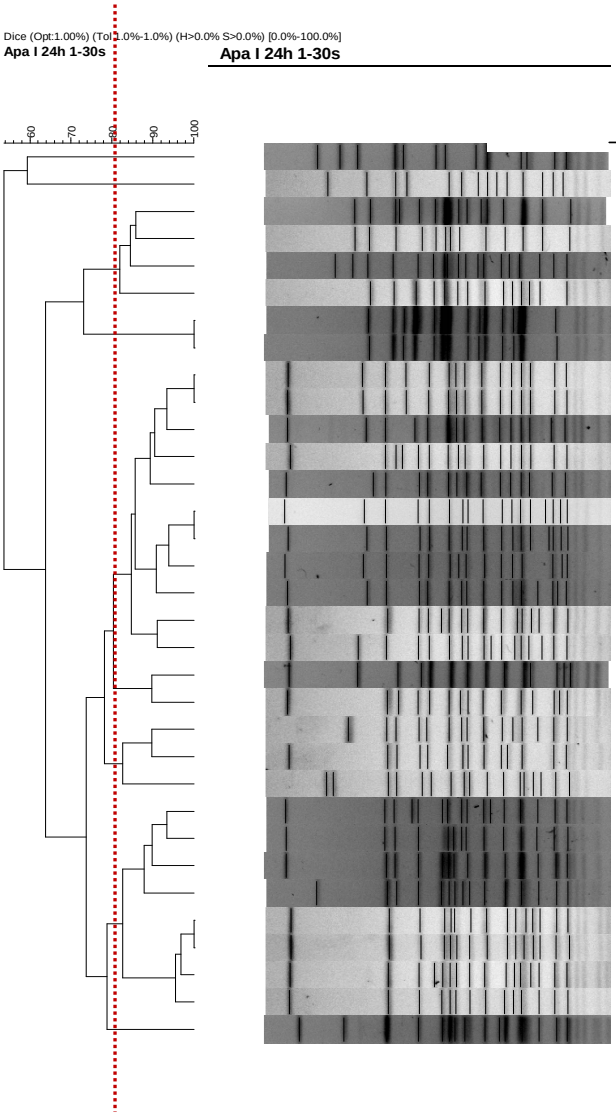
MIRLNO	Region	SOURCE TYPE	ESBL	AmpC
2008LSM165	N	Urine	Y	Y
2008MSH142	N	Others	Y	N
2008KTC138	N	Blood	N	N
2008NCK208	S	Blood	N	Y
2008KSM093	S	Urine	Y	N
2008TRS127	N	Wound	Y	N
2008CGK198	S	Blood	Y	Y
2008LBA155	N	Resp.	Y	N
2008GTC088	M	Urine	Y	N
2008SLC173	M	Blood	Y	N
2008SLC139	M	Urine	Y	N
2008SLC110	M	Urine	N	N
2008SLC148	M	Blood	Y	N
2008SLC113	M	Resp.	Y	N
2008SLC085	M	Wound	Y	N
2008NCK173	S	Blood	Y	N
2008NCK226	S	Blood	Y	N
2008MNC142	E	Urine	Y	N
2008SLC167	M	Blood	Y	N
2008SLC179	M	Blood	Y	N
2008SLC156	M	Blood	Y	N
2008SCM164	M	Urine	Y	N
2008ZAT095	M	Urine	Y	N
2008SCM232	M	Blood	N	Y
2008SLC078	M	Urine	N	Y
2008GTC062	M	Urine	N	Y
2008GTC166	M	Resp.	N	Y
2008NCK118	S	Blood	Y	N
2008SLC212	M	Blood	N	Y
2008LSM239	N	Blood	Y	Y
2008SCM179	M	Resp.	Y	Y
2008PHL156	E	Resp.	Y	Y
2008ZGP074	E	Blood	Y	Y
2008MNC416	E	Urine	Y	Y
2008CHC106	M	Urine	Y	N
2008MNC162	E	Urine	Y	N
2008MSH105	N	Urine	N	Y
2008KSM112	S	Urine	N	N

Figure 5. Dendrogram of 34 nosocomial carbapenem-resistant *Pseudomonas aeruginosa* from 2004, 2006, and 2008.



MIRLNO	SOURCE	Region	MBL	IMP	IMP2-M	MEM	MEM2-M
2004CSM155	Resp.	M	-	R	16	R	16
2006YTM128	Other	N	-	R	16	R	>16
2004ZAT092	Resp.	M	-	R	16	R	16
2006SCM155	Resp.	M	-	R	16	R	16
2006VGK164	Wound	S	-	I	8	R	16
2008LSM136	Urine	N	-	S	2.00	S	<=1.00
2008NCK165	Resp.	S	-	R	>16.00	S	4
2004NCK112	Resp.	S	-	R	16	S	4
2008HLH401	Resp.	S	-	R	>16.00	I	8
2008HLH403	Resp.	S	-	R	>16.00	R	>8.00
2008VGK091	Resp.	S	-	R	>16.00	I	8
2006ZGP165	Resp.	E	-	R	>16	S	4
2004LSM178	Resp.	N	-	I	8	S	4
2008ZGP077	Blood	E	-	R	16.00	I	8
2006KTC107	Resp.	N	-	R	>16	R	16
2006SCM213	Blood	M	-	R	16	S	4
2008SCM241	Blood	M	VIM-2	I	8.00	S	4
2008SCM412	Resp.	M	VIM-2	I	8.00	S	2
2006ZAT057	Urine	M	VIM-2	I	8	I	8
2004SCM054	Urine	M	VIM-2	R	16	R	16
2006SCM094	Resp.	M	VIM-2	I	8	S	4
2008NCK170	Resp.	S	-	R	16.00	I	8
2006TZH125	Wound	N	-	R	>16	S	4
2008HLH090	Urine	S	-	R	>16.00	S	4
2004CGK170	Resp.	S	-	R	16	S	4
2008CGK154	Urine	S	-	I	8.00	R	>8.00
2008KSM097	Wound	S	-	S	4.00	S	<=1.00
2008KTC160	Urine	N	-	I	8.00	S	2
2006MKS103	Urine	S	-	R	16	S	4
2006NCK094	Other	S	-	R	16	I	8
2004HLH150	Resp.	S	-	R	16	S	4
2006KTC155	Blood	N	-	R	16	R	>16
2006MKS064	Resp.	S	-	I	8	S	1
2006CGK172	Urine	S	-	R	16	S	4

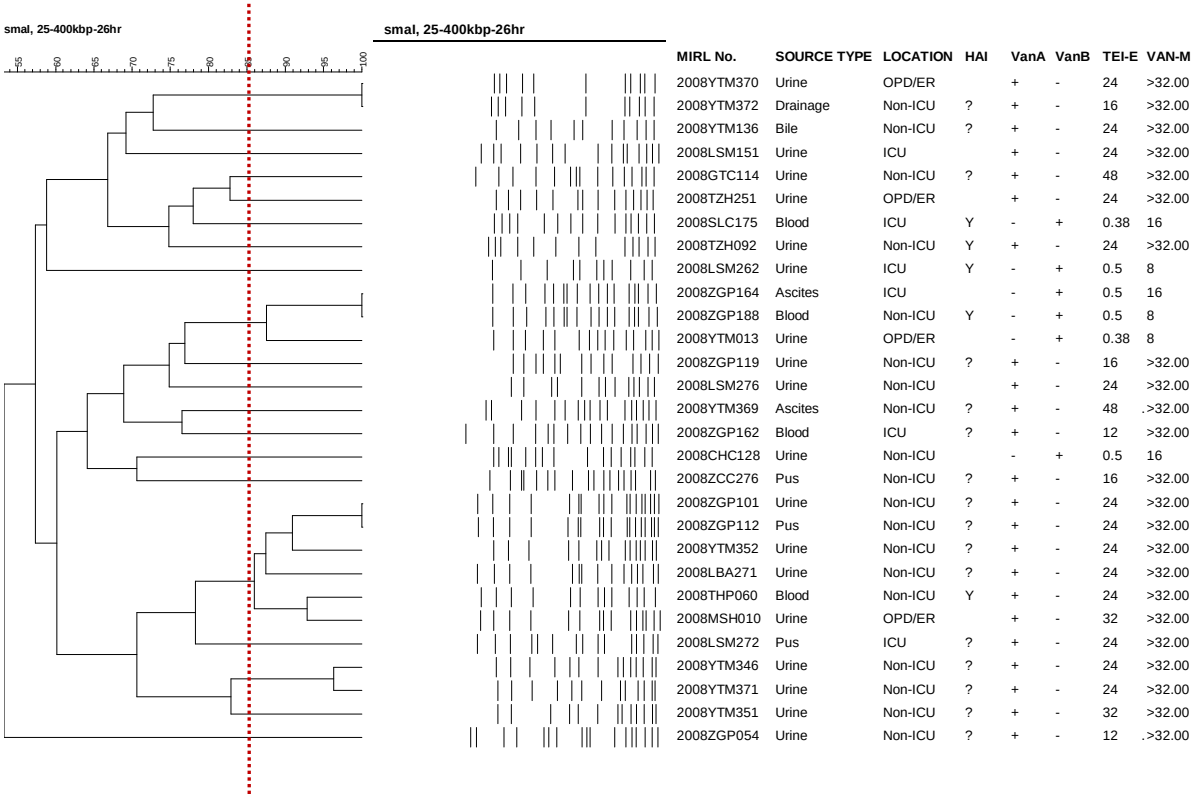
Figure 6. Dendrogram of *Apa*I digested chromosomal DNA of 33 nosocomial carbapenem-resistant *Acinetobacter baumannii* from different hospitals in 2008.



MIRLNO	TSAR	SOURCE TYPE	ISAba1	OXA-gene group	ISF/ OXA51R	ISF/ OXA23R
2008SLC052	VI	Urine	Pos	23*,51*	Neg	Pos
2008SLC168	VI	Blood	Pos*	51	Neg	Pos*
2008SLC053	VI	Resp.	Pos	23*,51	Neg	Pos
2008SLC120	VI	Urine	Pos	23*,51	Neg	Pos
2008MSH141	VI	Others	Pos	23*,51*	Neg	Pos
2008SLC094	VI	Resp.	Pos	23*,51	Neg	Pos
2008SCM202	VI	Blood	Pos	23*,51	Neg	Pos
2008SCM234	VI	Blood	Pos	23*,51	Neg	Pos
2008SLC174	VI	Blood	Pos	23*,51	Neg	Pos
2008SLC222	VI	Blood	Pos	23*,51	Neg	Pos
2008CSM151	VI	Blood	Pos	23*,51*	Neg	Pos
2008ZCC078	VI	Urine	Pos	23*,51*	Neg	Pos
2008CSM207	VI	Blood	Pos	23*,51	Neg	Pos
2008CGK102	VI	Urine	Pos	24*,51*	Neg	Neg
2008CSM210	VI	Blood	Pos	51	Pos*	Neg
2008NCK081	VI	Blood	Pos*	51*	Pos*	Neg
2008CSM242	VI	Blood	Pos	23*,51	Neg	Pos
2008VGK171	VI	Others - SBS	Pos	24*,51	Neg	Neg
2008ZCC152	VI	Others - SBS	Pos	23*,51	Neg	Pos
2008SCM414	VI	Urine	Pos*	23*,51	Pos*	Pos*
2008VGC148	VI	Others - SBS	Pos	23*,51*	Neg	Pos
2008VGK163	VI	Others-Left JP	Pos	24*,51*	Neg	Neg
2008YTM172	VI	Resp.	Pos	51*	Pos*	Neg
2008SLC154	VI	Blood	Pos*	51	Pos*	Neg
2008SCM152	VI	Resp.	Pos	23*,51*	Neg	Pos
2008SCM156	VI	Resp.	Pos	23*,51	Neg	Pos
2008SCM230	VI	Blood	Pos	23*,51	Neg	Pos
2008SCM167	VI	Resp.	Pos	23*,51	Neg	Pos
2008SLC111	VI	Resp.	Pos	23*,51	Neg	Pos
2008SLC132	VI	Resp.	Pos	23*,51	Neg	Pos
2008SLC103	VI	Resp.	Pos	23*,51*	Neg	Pos
2008SLC155	VI	Blood	Pos	23*,51	Neg	Pos
2008SCM413	VI	Resp.	Pos	23*,51	Neg	Pos

Figure 6 legend. ISF/Oxa51R, PCR using forward primer of IS *Aba1* and reverse primer of Oxa-51. ISF/Oxa23R, PCR using forward primer of IS*Aba1* and reverse primer of Oxa-23. *Indicates that PCR products have been sequenced.

Figure 7. Dendrogram of *Sma*I digested chromosomal DNA pulsed field gel electrophoresis pattern of 30 vancomycin resistant *Enterococcus faecium*.



MIRL No.	SOURCE TYPE	LOCATION	HAI	VanA	VanB	TEI-E	VAN-M
2008YTM370	Urine	OPD/ER		+	-	24	>32.00
2008YTM372	Drainage	Non-ICU	?	+	-	16	>32.00
2008YTM136	Bile	Non-ICU	?	+	-	24	>32.00
2008LSM151	Urine	ICU		+	-	24	>32.00
2008GTC114	Urine	Non-ICU	?	+	-	48	>32.00
2008TZH251	Urine	OPD/ER		+	-	24	>32.00
2008SLC175	Blood	ICU	Y	-	+	0.38	16
2008TZH092	Urine	Non-ICU	Y	+	-	24	>32.00
2008LSM262	Urine	ICU	Y	-	+	0.5	8
2008ZGP164	Ascites	ICU		-	+	0.5	16
2008ZGP188	Blood	Non-ICU	Y	-	+	0.5	8
2008YTM013	Urine	OPD/ER		-	+	0.38	8
2008ZGP119	Urine	Non-ICU	?	+	-	16	>32.00
2008LSM276	Urine	Non-ICU		+	-	24	>32.00
2008YTM369	Ascites	Non-ICU	?	+	-	48	.>32.00
2008ZGP162	Blood	ICU	?	+	-	12	>32.00
2008CHC128	Urine	Non-ICU		-	+	0.5	16
2008ZCC276	Pus	Non-ICU	?	+	-	16	>32.00
2008ZGP101	Urine	Non-ICU	?	+	-	24	>32.00
2008ZGP112	Pus	Non-ICU	?	+	-	24	>32.00
2008YTM352	Urine	Non-ICU	?	+	-	24	>32.00
2008LBA271	Urine	Non-ICU	?	+	-	24	>32.00
2008THP060	Blood	Non-ICU	Y	+	-	24	>32.00
2008MSH010	Urine	OPD/ER		+	-	32	>32.00
2008LSM272	Pus	ICU	?	+	-	24	>32.00
2008YTM346	Urine	Non-ICU	?	+	-	24	>32.00
2008YTM371	Urine	Non-ICU	?	+	-	24	>32.00
2008YTM351	Urine	Non-ICU	?	+	-	32	>32.00
2008ZGP054	Urine	Non-ICU	?	+	-	12	.>32.00

Figure 7 legend. HAI, Y indicates HAI determined by hospital infection control staff; ? indicates isolates not identified as HAI by hospital infection control staff but were cultured >2 days after admission, thus are possible HAI cases. MIC (μg/mL) of teicoplanin (TEI) and vancomycin (VAN) are shown. Isolates with >80% similarity (dashed vertical line) are considered probably or closely related. Isolates with indistinguishable PFGE pattern are considered identical.