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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(multidrug resistant bacteria)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，是近年來的重大全球公衛危機。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。

控制多重抗藥菌需要首先了解各地區多重抗藥菌的盛行率、流行病學及其抗藥機制。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。此計劃去年已調查了第六期 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)，也提供技術協助，調查 TSAR 醫院之群突發案件及不尋常抗藥菌

萬古黴素是治療嚴重 MRSA 感染的首選。然而，菌株對 vancomycin 感受性降低而導至治療失敗之個案日增，使得 daptomycin 的使用持續增加。 Daptomycin 是一個新的鈣質依賴性抗生素。台灣金黃色葡萄球菌中對 daptomycin 之抗敏性還不清楚。以往的研究顯示，台灣 MRSA 屬於 4 個主要族群，但因 TSAR 菌株中這 4 個主要族群的臨床資料有限。碳青黴烯類是治療感染對廣泛性乙內醯氨具抗藥性大腸桿菌及克雷白氏肺炎桿菌(extended spectrum β-lactam resistant *E. coli* and *K. pneumoniae*)之最後一線藥物。近年來新興的碳青黴烯酶(carbapenemase)另人憂心。目前已有十幾種碳青黴烯酶被發現，對不同碳青黴烯類藥(carbapenems)也具不同抗藥程度，包括新型 NDM-1(新德里的金屬 β-內酰胺酶-1)，是全球最近最重視的抗藥問題。台灣面臨令一個重要感染控制挑戰是 carbapenem-resistant 包氏不動桿菌(CRAB)。由於常規 TSAR 調查每 2 年才進行一次，為能較即時偵測新興抗藥菌之產生，2009 年底對幾家 TSAR 醫院進行以上

carbapenem 抗藥革蘭氏陰性菌之收集。因此，此計畫今年(2010)研究主要對象為對最後線抗生素感受性降低或具混合抗藥性的菌，包含對萬古黴素或對 daptomycin 感受性降低或具混合抗藥性的金黃色葡萄球菌。為增加 MRSA 臨床流行病學之了解，亦對 MRSA 個案進行問卷調查。因對 carbapenem 具抗藥性之革蘭氏陰性菌為一新興抗藥問題，亦特別調查抗 carbapenem 大腸桿菌、克雷白氏肺炎桿菌、及鮑氏不動桿菌。

我們調查了 292 株 *S. aureus* 的 daptomycin MIC，包括 231 株 MRSA 和 61 株 MSSA。MRSA 跟 MSSA 的 daptomycin MIC_{50} (抑制 50%的菌的藥濃度都是 0.5 ug/mL，而 MIC_{90}s 的 MRSA 和 MSSA 分別為 1 和 0.5 ug/mL (1 ug/mL 以下為具感受性)。有一株 MRSA 之 daptomycin MIC 為 2 ug/mL，所以 MRSA 對 daptomycin 之抗藥性還很低(0.4%，1 / 231）。但使用 Etest 方法的則有 4 株菌對 daptomycin 不具敏感性(3.6%，4 / 110）。我們也對來自一位 daptomycin 治療失敗的個從血液培養出的 8 株 MRSA(CGK1-8)進行基因型研究。這 8 株菌的基因型皆一樣，CGK1 - CGK5 的 daptomycin MIC 為 0.75 ug/mL 以下，但 CGK6-8 提高到 4 ug/mL。 CGK7 還對 vancomycin 產生中度抗抗藥性(MIC 3 ug/mL)，我們也在 CGK6-8 的 mprF 基因(一個會影響細菌表面電荷的基因)中發現一個單點突變導致 L431F 之改變，這是過去沒被報告過的突變點。

有關 MRSA 個案臨床資料調查已完成 149 份，包含 SCC*mec* II、III、IV、V 型的 MRSA 分別為 19(12.8%)、51(34.2%)、28(18.8%)及 51(34.2%)。感染 SCC*mec* IV 及 V 個案之年齡較為年輕，小兒病人分別佔了 21.4%及 35.3%。SCC*mec* II 及 III MRSA 感染之個案病人大多為住院病人，但感染 SCC*mec* V MRSA 之病人則多為門診病人(43.1%)，而且只有一位病人因此而住院。大多數的 SCC*mec* II(94.7%)及 III(84.3%)個案皆有潛伏疾病，SCC*mec* V 個案中，只有 29.4%有其它潛在疾病。在這些調查個案中，有 9 例(6%)因感染 MRSA 而死亡，大多病人皆康復，但 SCC*mec* II 病患中有 21%死亡，因各 SCC*mec* 型別的個案不多，還需更多個案資料才能確認初步分析結果。

我們調查了從去年 9 月至今年 6 月，來自 3 家醫院的 23 株 carbapenem-resistant *E. coli* (簡稱 CR-eco)及 48 株 carbapenem-resistant *K. pneumoniae* (簡稱 CR-kpn)，這 71 株菌共來自 53 個病人，病人多為高年齡群(平均年齡為 71 歲)。其中有些菌僅對 ertapenem 有抗藥性，但也有菌對 ertapenem, imipenem 及 meropenem 皆有抗藥性，大多 CR-eco 菌(21 株, 91.3%)帶有 AmpC 抗藥基因，其中 5 株同時帶有 ESBL 抗藥基因，在 CR-kpn 菌中，則有 25 株帶有 ESBL，帶有 AmpC 抗藥基因的有 35 株，其中 19 株同時帶有 ESBL。CR-kpn 菌則有 3 株(來自同一病人)帶有 IMP-8 carbapenemase (碳青黴烯酶)，此 3 株菌並同時帶有 CTX-ESBL。這些 71 株菌皆未測到新型 NDM-1(新德里的金屬 β-內酰胺酶-1)抗藥基因。經 PFGE 測試結果顯示，CR-eco 及 CR-kpn 菌群中皆含有同一型或親聯性高的菌群，表示這些抗藥菌在不同病人中互傳。

從 2009 年 9 月至 2010 年 3 月，我們亦從 6 家醫院收集到來自 421 個 ICU 病人的 587 株鮑氏不動桿菌(*A. baumannii*，簡稱為 AB)，其中有 472 株為抗 carbapenem 菌 (CRAB)，當只用每個病人之第一株 AB 菌進行分析時，CRAB 比率為 78.1%。此 6 家醫院 ICU 病人 CRAB 比率差異很大，從 56.7%到 99%。這些 472 株 CRAB 皆未測到新型 NDM-1 抗藥基因。從這些 CRAB 菌挑出 108 株菌進行了 PFGE 測試，結果顯示，這 6 家醫院的 CRAB 並不相同，但每家醫院中各有型別一樣之菌，有些菌是來自不同病人不同時間分離出的菌，同一型別之 CRAB 在超過一個月後，甚至可在 6 個月後仍可在不同病人被分離出，表示 CRAB 可長期維持在各醫院環境中，在不同病人間持續傳播。

今年研究結果顯示，雖然台灣的金黃色葡萄球菌對新的後線抗生素 daptomycin 的抗藥性仍低(< 1%)，但已從一個 daptomycin 治療失敗的個案血液分離出的 8 株 MRSA 中，找到對 daptomycin 有抗藥性的菌，其中有一株對 vancomycin 也產生中度抗藥性。因此當使用 daptomycin 或 vancomycin 治療嚴重 MRSA 感染個案時，應特別觀測個案之 MRSA 是否逐漸對 daptomycin 或 vancomycin 產生抗藥性。對 4 種 SCC*mec* II、III、IV、V 型的 MRSA 個案之分析，顯示病人群有差異，SCC*mec* V 型的病人族群最年輕，較無潛在疾病，且多為社區感染。感染 MRSA 的平均死亡率為 6%，但 SCC*mec* II 型的個案死亡率為 21%，此為初步分析結果，尚需收集更多個案加以確認。本研究亦發現同一型別的 CR-eco 及 CR-kpn 已在不同時間及不同病人分離到，表示這些多重抗藥菌以在醫院內散播。此外，因部份 CR-eco 及 CR-kpn 僅對 ertapenem 有抗藥性，但對 imipenem 及 meropenem 無抗藥性，如醫院未使用 ertapenem 在測試對 carbapenem 的抗敏性，可能不會偵測到對 carbapenem 有低程度的抗藥菌。TSAR 過去資料顯示，台灣自加護病房分離出的 AB 菌中，CRAB 的比例逐年上升(2004 年: 15%，2006 年: 45%，2008 年: 66%)，本研究發現已高達 78%。PFGE 結果顯示，6 家醫院各有些 CRAB 是來自不同病人不同時間分離出相同型別的菌，表示 CRAB 在各醫院不同病人中持續傳播。總結以上研究結果，顯示台灣多重抗藥菌及對最後線抗生素具抗藥性之細菌持續增加中，因此，建議對高危險病人群及特別病房個案積極進行多重抗藥菌之監測，並加強院內感控措施之落實及管控，以控制多重抗藥菌之擴散及院感之發生。

關鍵詞：多重抗藥菌、抗 daptomycin 之 MRSA、抗 carbapenem 大腸桿菌、抗 carbapenem 克雷白氏肺炎桿菌、抗 carbapenem 鮑氏不動桿菌。

英文摘要

English Abstract

Background and Purpose

Multidrug resistant (MDR) bacteria pose significant threat to the global public health due to diminishing treatment choices and poorer treatment outcomes. Strategies for the control of MDR bacteria require first an understanding of their prevalence and epidemiology within a local region and their mechanisms of resistance. In the hospital, ICU patients are exposed to more intense antimicrobial use and invasive procedures. In addition, MDR bacteria can persist in the hospital environment and be transmitted between patients to cause nosocomial infections. Therefore, isolates from ICU and nosocomial infections are usually the most resistant.

This two and half year project was carried out to study the mechanism of resistance and molecular epidemiology of MDR bacteria collected in different rounds of the Taiwan Surveillance of Antimicrobial Resistance (TSAR). TSAR is conducted by the National Health Research Institutes (NHRI). Since 1998, TSAR survey has been performed every 2 years on multiple hospitals from the 4 regions of Taiwan. For this project in 2008 and 2009, the following MDR nosocomial isolates have been studied: methicillin resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactam non-susceptible *Escherichia coli* and *Klebsiella pneumoniae*, carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (CRAB), and vancomycin-resistant enterococci.

Vancomycin (VAN) is the main treatment choice for serious MRSA infections. However, treatment failures due to strains with reduced susceptibility to VAN have been reported and the use of daptomycin, a new calcium dependent lipopeptide agent, has increased in recent years. The prevalence of daptomycin nonsusceptibility (DAP-NS) in *S. aureus* in Taiwan is currently unknown. Previous studies have shown MRSA in Taiwan belong to 4 major clones based on pulsed field gel electrophoresis (PFGE) pulsotypes and genetic profile. However, clinical data on these 4 major clones in TSAR isolates is limited. Carbapenems are the last line agents for treating infections caused by MDR *E. coli* and *K. pneumoniae*. Another MDR bacterial pathogen, CRAB, has become highly prevalent in Taiwan in recent years. The recent emergence of carbapenemase NDM-1 producing Gram-negatives in different species and countries indicated the ability of some MDR bacteria or resistance determinants for rapid spread. Accurate and timely detection of isolates with emerging resistance to these last line treatment choices is a key step in preventing their spread. Because routine TSAR survey is conducted every 2 years, we

carried out a special TSAR collection on a few hospitals for the three MDR Gram-negative species.

Therefore, for this year, the following MDR bacterial species were targets of study. 1. Daptomycin and vancomycin non-susceptible *S. aureus*, 2. MRSA case retrospective review on risk factors, disease spectrum and treatment outcome in the 4 MRSA clones, 3. Carbapenem resistant *E. coli* and *K. pneumoniae*, and 4. Carbapenem resistant *A. baumannii* (CRAB).

Methods

We first established an in-house broth microdilution (BMD) test protocol for determining daptomycin MIC in TSAR *S. aureus*. Etest was also performed on a portion of the isolates. The association of vancomycin and daptomycin levels was also determined. Daptomycin resistant strains from a patient who failed daptomycin therapy were also subjected to genotyping including staphylococcus cassette chromosome *mec* (*SCCmec*) typing, Panton-Valentine leukocidin toxin genes (*pvl*) detection and multilocus sequence typing (MLST). Population analysis profiles for daptomycin and vancomycin were also performed. Sequencing for mutation in *mprF*, a gene associated with daptomycin nonsusceptibility was also performed. All *E. coli* and *K. pneumoniae* suspected to be non-susceptible to carbapenem were subjected to extended spectrum β-lactamase (ESBL) confirmatory test and modified Hodge test for the presence of metallo-β-lactamase. Multiplex PCR was used to detect the genes encoding ESBL (SHV and CTX-M type), plasmid-mediated class C AmpC β-lactamases (DHA and CMY), and carbapenemases (NMC, SME, IMI, KPC, GIM, SPM, NDM-1, and OXA-48). All CRAB isolates were subjected to PCR for NDM-1 also. PFGE was used to determine strain relatedness on selected strains.

Results.

Daptomycin and vancomycin non-susceptible *Staphylococcus aureus*.

A total of 292 non-duplicate *S. aureus* isolates were tested for daptomycin MIC including 231 MRSA and 61 methicillin-susceptible *S. aureus* (MSSA). The MIC_{50s} of MRSA and MSSA were the same (0.5 μg/mL), and the MIC_{90s} of MRSA and MSSA were 1 and 0.5 μg/mL, respectively. Nonsusceptibility (MIC > 1 ug./mL) to daptomycin was found in one MRSA isolate (0.4%, 1/231) (MIC 2 μg/mL). By Etest, the MIC_{50s} and MIC_{90s} were slightly higher, at 0.75 and 0.5 μg/mL, and 1 and 0.5 μg/mL for MRSA and MSSA, respectively. There were 4 daptomycin nonsusceptible MRSA isolates by Etest (3.6%, 4/110), albeit with MIC of 1.5 μg/mL. By BMD, 3 of these 4 isolates had MIC of 1 μg/mL and the other had MIC of 2 μg/mL. Higher daptomycin MIC distribution was seen in isolates with higher vancomycin MIC.

Genotypic study on 8 consecutive MRSA blood culture isolates recovered over an 8 month period was carried out. The isolates (CGK1-8) were from a patient who failed daptomycin therapy. These 8 isolates had indistinguishable PFGE pattern and genetic profile. Daptomycin MIC in CGK1-CGK5 were $\leq$ 0.75 μg/mL but increased to 4 μg/mL in CGK6-CGK8. CGK7 also developed intermediate resistance to vancomycin (MIC 3 μg/mL) while the other isolates had MIC of 1-2 ug/mL. Population analysis profile (PAP) performed for daptomycin showed a distinct increased population of CGK6-CGK8 able to grow in daptomycin concentrations of > 2 μg/mL. PAP for vancomycin showed that CGK7 fit the hVISA criteria. Sequencing of *mprF*, the gene encoding lysyl-phosphatidylglycerol synthase and translocase, revealed a single-point mutation (C to T) resulting in change of leucine to phenylalanine (L431F) in CGK6-CGK8 while no mutation was found in all 8 isolates in other sites previously reported by other research groups.

MRSA case study.

A total of 191 MRSA case reports from 4 hospitals were reviewed by each hospital infectious disease physician. Because of the high correlation of PFGE and SCC*mec* type, analysis was made based on SCC*mec* types on 149 cases with complete history including 19 (12.8%), 51 (34.2%), 28 18.8%), and 51 (34.2%) MRSA strains carrying SCC*mec* II, SCC*mec* III, IV and V, respectively. There were more pediatric patients in the SCC*mec* type IV (21.4%) and V (35.3%) group compared to SCC*mec* II (10.5%) and III (3.9%) groups. The majority of the patients in the SCC*mec* II and III groups were inpatients. In contrast, 22 (43.1%) of the patients in the SCC*mec* V group presented as outpatients, and only 1 was admitted. Most patients in the SCC*mec* II (18, 94.7%) and III (43, 84.3%) groups had underlying diseases whereas significantly fewer patients (15, 29.4%) in the SCC*mec* V group had underlying diseases ($p < 0.01$). More patients in the SCC*mec* II group had respiratory tract infections (RTI, 57.9%) and bloodstream infections (BSI, 52.6%), whereas skin and soft tissue infections (SSTI,19, 37.3%) and BSI (15, 29.4%) were the most common infections in the SCC*mec* III group, while SSTI was the most common infection in the SCC*mec* V group (66.7%). The majority of patients recovered (cured or improved) from the MRSA infections, ranging from 52.6% in the SCC*mec* II group to 88.2% in the SCC*mec* V group. Direct attributable death ranged from 3.6% in the SCC*mec* IV group to 21.1% in the SCC*mec* II group.

Carbapenem resistant (CR) *E. coli* and *K. pneumoniae*.

A special TSAR collection for *E. coli* and *K. pneumoniae* isolates suspected to be carbapenem (ertapenem, imipenem, or meropenem) resistant was conducted between

September 2009 and June 2010. A total of 71 isolates including 23 *E. coli* and 48 *K. pneumoniae* from 53 patients were studied. The average age of these patients was 71 yo (median 76 yo). Some isolates were resistant to ertapenem only, while some were resistant to all ertapenem, imipenem, and meropenem. The majority of the *E. coli* isolates carried bla_{AmpC} (21 isolates, 91.3%) (from 16 patients), 20 of which were CMY-type. Five isolates positive for bla_{ESBL} CTX-M were also positive for bla_{AmpC} (1 DHA-type and 4 CMY-type). In the 48 *K. pneumoniae* isolates, 4 were positive for SHV-type bla_{ESBL} , 25 were positive for CTX-M type bla_{ESBL}. DHA and CMY -type bla_{AmpC} were found in 23 and 12 of *K. pneumoniae* isolates, respectively. Nineteen isolates carried both bla_{ESBL} (18 CTX-M type and 1 SHV-type).

No carbapenemase was detected in *E. coli*. Three 3 *K. pneumoniae* isolates were positive for class B carbapenemase IMP-8, all 3 were all from the same patient isolated 13 days apart and shared identical PFGE pattern, and were all positive for CTX-M also. PCR for the newly emerged $bla_{\text{NDM-1}}$ (New Delhi metallo-β-lactamase gene) was also performed on all 71 isolates and none was detected. PFGE indicated that 4 *E. coli* isolates from 3 patients recovered a month apart shared indistinguishable PFGE pattern. In *K. pneumoniae*, 3 clusters of isolates from 1 hospital showed >80% similarity in PFGE pattern. These isolates were either from the same patient recovered from different body sites or from the same specimen type on different days, or from different patients. Isolates with indistinguishable pattern were found within each cluster.

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) study

Between September 2009 and March 2010, a total of 587 isolates from 421 patients were collected from ICU of 6 hospitals, of which 468 were CRAB. When only the first isolate of each patient was used for analysis, carbapenem resistance was 78.1% overall but differences in CRAB rates were noted between the hospitals (56.7% to 99%). PCR for $bla_{\text{NDM-1}}$ was performed on all 468 CRAB but did not detect any positive ones. PFGE performed on 131 CRAB showed that isolates from each hospital were distinct from isolates of other hospitals but there were several pairs and clusters of isolates from different patients within each hospital having indistinguishable or closely related PFGE patterns, including some isolates recovered more than 1 month apart, with some even at over 6 month span, indicating that CRAB can persist for a long time in the hospital environment and clonal spread within each hospital has occurred.

Conclusions.

Although we found that the majority of MRSA and MSSA in Taiwan were susceptible to daptomycin (DAP), DAP-nonsusceptible MRSA isolates were recovered

from a patient who failed DAP therapy and vancomycin heterogeneous intermediate resistance also developed in one MRSA. Therefore, careful monitoring of DAP and VAN reduced susceptibility is warranted in patients with severe MRSA infections who are being treated with either one of these 2 agents. Preliminary analysis on MRSA cases indicated that significant differences exist in the patients infected by the 4 major MRSA clones in Taiwan. MRSA strains harboring SCC*mec* V are considered community-MRSA in Taiwan, and our data indicated that patients in this group are younger and fewer of them had underlying diseases and other risk factors. The overall mortality attributable to MRSA infections in the case study patients was 6.0% (9 patients) but patients in the SCC*mec* II group had higher mortality (21.1%). Because of the small sample size in SCC*mec* II group, more cases and multivariate analysis are needed to confirm these findings.

The finding of multiple carbapenem-resistant *E. coli* and *K. pneumoniae* isolates with identical or highly similar PFGE patterns from different patients indicated that clonal spread has occurred. Since some of these CR isolates were resistant to ertapenem only, and since not all hospitals use ertapenem to screen for carbapenem-resistance, *E. coli* and *K. pneumoniae* with low level carbapenem resistance may be missed in some hospitals. The majority of the patients were elderly (>70 yo). The prevalence of CRAB has increased dramatically in the last few years in Taiwan. TSAR VI (2006) data indicated that 66% of *A. baumannii* from ICU patients were CRAB, compared to 15% in 2004 and 45% in 2006. The present study found rates of CRAB from ICU of 6 hospitals to be 78.1% and clonal spread of CRAB within each hospital also occurred. We conclude that active surveillance for carbapenem resistance in *Enterobacteriaceae* and *A. baumannii* in high risk group patients is warranted. Enforcement of stringent infection control measures in high risk areas of the hospitals is urgently needed.

Key Words: Multidrug resistant bacteria, methicillin resistant *Staphylococcus aureus* (MRSA), carbapenemase, carbapenem resistant *Escherichia coli*, carbapenem resistant *Klebsiella pneumoniae*, carbapenem resistant *Acinetobacter baumannii*.

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[WHO 2001; Livermore 2006]。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[Lodise et al., 2005; Lee et al., 2007，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)。

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[Ho et al., 1999; McDonald et al., 2004; Lauderdale et al., 2004]。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，故是一個好的研究資源[Chen et a., 2005 & 2009; Yang et al., 2009; Sheng et al., 2010]。TSAR 資料顯示，對廣泛性乙內醯氨具抗藥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)具抗藥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藥性的金黃色葡萄球菌(MRSA)為最常見的多重抗藥菌。

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，這些基因是導致多重抗藥性菌之主要原因。對廣泛性乙內醯氨具抗性之腸桿菌及克雷白氏肺炎桿菌(extended spectrum β-lactam resistant *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。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有抗藥性的腸桿菌[Lee et al., 2008]，是一需仔細監測的新興抗藥菌。近年來新興的乙醯胺酶，碳青黴烯酶(carbapenemase)更另人憂心。目前已有十幾種碳青黴烯酶被發現，除可水解所有青黴素類及頭孢子素類藥物，對不同碳青黴烯類藥(carbapenems)也具不同抗藥程度[Livermore et al., 2006; Queenan & Bush 2008\]，加上這些碳青黴烯酶基因常位於具多重抗藥基因的質體上，因此碳青黴烯類抗藥菌治療選擇非常有限。帶有新型碳青黴烯酶NDM-1(新德里的金屬β-內酰胺酶-1) (New Delhi metallo-β-lactamase)的不同腸桿菌，在印度、巴基斯，英國及世界不同地區已發現，是全球最近最重視新興抗藥菌。

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* (*SCCmec*)，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，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。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 *SCCmec* types II 或 III，而 C-MRSA 則大多具 *SCCmec* type IV。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。

此計畫為兩年半之連續型計畫，於 2008 年七月開始，今年為最後一年。此計畫之總目標為對 TSAR I (1998) 至 TSAR VI（2008）中由各 TSAR 醫院之院感菌種及部份非院感常見多重抗藥菌種，分年度對不同抗藥菌進行抗藥基因及菌株間親聯性之研究。此計畫 2008-2009 年已完成 TSAR 多種抗藥菌之調查，包含：院感及非院感抗甲

氧苯色葡萄球菌(MRSA)、對 carbapenem 具抗藥性之院感綠膿桿菌及鮑氏不動桿菌(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、院感及非院感對萬古黴素具抗藥性之腸球菌(VRE)。2009 年亦開始偵測新興抗藥菌，調查 *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)。

今年(2010)研究主要對象為對最後線抗生素具抗藥性的菌，包含對萬古黴素或對 daptomycin 感受性降低或具混合抗藥性的金黃色葡萄球菌。為增加 MRSA 臨床流行病學之了解，亦對 MRSA 個案進行問卷調查。另外，因為對 carbapenem 具抗藥性之革蘭氏陰性菌，是非常重要的新興抗藥問題，亦特別調查抗 carbapenem 大腸桿菌、克雷白氏肺炎桿菌、及鮑氏不動桿菌。此研究計畫調查不同期間院感之常見多重抗藥菌種及新興抗藥菌，以了解這些抗藥菌之抗藥機制及分子流行病學及其在不同醫院分佈情況，並追蹤其演變。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。此計畫結果顯示抗藥菌之防治與控制，除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，更需要強制實施感控措施，以減少多重抗藥菌之擴散。

(二) 材料與方法 (Materials and Methods)

■ **研究對象：**

今年(2010)研究主要是以對最後線抗生素具抗藥性的菌，包含:

1. 對萬古黴素或對 daptomycin 感受性降低或具混合抗藥性的金黃色葡萄球菌 (daptomycin and vancomycin heterogeneous resistant *S. aureus*)。
2. 另外，為增加 MRSA 臨床流行病學之了解，亦對 MRSA 個案進行問卷調查。
3. 對 carbapenem 具抗藥性之大腸桿菌及克雷白氏肺炎桿菌 (carbapenem resistant *E. coli* and *K. pneumoniae*)。
4. 對 carbapenem 具抗藥性之鮑氏不動桿菌(Carbapenem-resistant *Acinetobacter baumannii* ，CRAB)。

■ **菌株及個案來源：**

1. 對萬古黴素或對 daptomycin 感受性降低或具混合抗藥性的金黃色葡萄球菌調查對象是從過去幾期 TSAR 中挑選不同 vancomycin MIC 之菌，以及醫院特別收集的 daptomycin 治療失敗個案之不同時間分離出的菌株。
2. MRSA 臨床流行病學之調查，則是針對四家醫院第三至第六期 TSAR 所收集到的菌株個案，進行問卷調查。
3. 對 carbapenem 具抗藥性之大腸桿菌及克雷白氏肺炎桿菌,則是特別邀請六家 TSAR 醫院特別收集任何疑是具 carbapenem 抗藥性之菌，收菌期間為 2009 年底至 2010 年前六個月。
4. 對 carbapenem 具抗藥性之鮑氏不動桿菌(CRAB) ，則是請同樣六家 TSAR 醫院，收集從 ICU 病人分離出的 AB 菌，收菌期間為 2009 年底至 2010 年三月。

■ **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，並挑選部份 MRSA 進行 Modified Population Analysis Profile (PAP)測試以找 hVISA (heterogeneous vancomycin intermediate *S. aureus*) 、ESBL confirmatory test 及 Modified Hodge Test [for metallo-β-lactamase (MBL) detection]表現型測試(phenotypic detection)、並使用 PCR 與 DNA sequencing 偵測不同 β-lactamases (ESBL, AmpC, and carbapenemase)之基因、並使用脈衝電泳法(Pulsed-Field Gel Electrophoresis,簡稱 PFGE)調查探討菌株之親聯性。實驗方法簡述如下：

A. 抗藥測試 (Antimicrobial susceptibility test). 抗藥測試除使用美國 Clinical and Laboratory Standards Institute [CLSI, 2010)]之 disk diffusion 及 broth microdilution

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。

B. 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[McDougal et al., 2003]，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 0.5μg/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。

C. *SCCmec* typing: 使用 multiplex PCR，測試 MRSA 之 *ccr* 及 *mec* 之型[Chongtrakool et al., 2006]。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*

D. PVL toxin gene detection: Primer 及 PCR 依照參考文獻[Ma et al., 2006]。

E. 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[Enright et al., 2000]。

F. Modified Population Analysis Profile (PAP)：將金黃色葡萄球菌實驗菌株接種於 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 及 daptomycin 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[Wootton et al., 2001]。

G. ESBL confirmatory test (產生 ESBL 菌之確定測試)：將 *E. coli* 及 *K. pneumoniae* 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 [CLSI 1010]。

H. 修改之 Hodge 測試法(Modified Hodge Test)：將調為 0.5 McFarland 濃度的 *E. coli* 及 *K. pneumoniae* 菌液均勻塗佈在 Mueller-Hinton Agar (MHA)上，乾燥後將含有 10 g 的 ertapenem 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 [CLSI 2010]。

I. ESBL、AmpC 、carbapenemase β-lactamase gene detection：使用單對及多對引子聚合酶連鎖反應法，偵測 β-lactamases 酵素基因。PCR primer 參考文獻[Monstein et al., 2007; Perez-Perez et al., 2002; Ellington et a., 2007; Queenan & Bush 2007]，不同基因及引子序列如表一及表二。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。

J. 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°C 20 分鐘接 80°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 µ 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。

K. *E. coli*、*K. pneumoniae*、*A. baumannii* 脈衝電泳法(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)專用緩衝溶液的限制酵素於水浴槽中作用 2 小時，再以 CHEF MAPPER 電泳系統 (Bio-Rad) 跑電泳後，將膠體放入 1　g/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。

L. 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。並以 Dice (Optimization:1.0%, Tolerance: 1.0%) 的條件製作 dendrogram 樹狀圖。亦參照 Tenover et al 之規則[Tenover et al., 1995]。

M. 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。統計學分析則使用 SPSS 軟體。

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

Part 1. Daptomycin and vancomycin non-susceptible *Staphylococcus aureus*

S. aureus is the most common Gram-positive pathogen in clinical medicine. Therapeutic choices for serious *S. aureus* infections can be limited, especially in methicillin-resistant S. aureus (MRSA) since MRSA are resistant to all available β-lactam agents. Vancomycin (VAN) has been the main treatment choice for serious MRSA infections. However, due to increasing reports of treatment failure in strains with reduced susceptibility or heterogeneous and/or intermediate resistance to VAN (hVISA and VISA), the use of daptomycin (DAP), a new calcium dependent lipopeptide agent [Baltz 2009], has increased in recent years. Although VAN and DAP differ in their modes of action, VAN-resistant *S. aureus* often display DAP-nonsusceptibility and vice versa.

1A. Prevalence of daptomycin nonsusceptibility (DAP-NS) in *S. aureus* in Taiwan.

Because the prevalence of daptomycin nonsusceptibility (DAP-NS) in *S. aureus* in Taiwan is currently unknown, we first determined the in vitro daptomycin activity against *S. aureus*. Antimicrobial susceptibility testing of DAP requires calcium supplements and currently only broth microdilution method (BMD) is recommended by the Clinical and Laboratory Standards Institute (CLSI). We first established an in-house BMD test protocol. Since Etest is also an accepted method even though it is not part of the CLSI protocol, we compared the DAP MIC by these two methods. The association of vancomycin and daptomycin levels was also determined.

1A-a. Test isolates. The isolates studied were from the Taiwan Surveillance of Antimicrobial Resistance (TSAR). A total of 292 non-duplicate isolates were tested including 190 from TSAR V (2006) and 102 from TSAR VI (2008), 62 of which were methicillin-susceptible *S. aureus* (MSSA) and 231 were MRSA. The isolates were from blood (n=52) and other sterile body sites (n=16), respiratory tract (n=62), and wound (n=137), with the reminder from miscellaneous sites and represented approximately 20% of the *S. aureus* in the TSAR V and VI (2006 & 2008) collections. The isolates were selected to include more vancomycin MIC > 0.5 μg/mL groups, so only 11 isolates (3.8%) had vancomycin MIC <=0.5 μg/mL, and the remaining isolates had vancomycin MIC of 1.0 μg/mL (n=216, 74.0%) and 2.0 μg/mL (n=65, 22.2%). For comparison, the vancomycin MIC <=0.5, 1.0, and 2.0 μg/mL groups comprised 6%, 84%, and 10% of the TSAR V and V *S. aureus* collections.

1A-b. MIC results by in house broth microdilution (BMD). The in vitro activity (MIC range, MIC_{50}, MIC_{90}) of daptomycin is shown in Table 1. By BMD, the MIC_{50s} of MRSA

(n=231) and MSSA (n=61) were the same (0.5 μg/mL), and the MIC_{90s} of MRSA and MSSA were 1 and 0.5 μg/mL, respectively. Nonsusceptibility to daptomycin was found in one MRSA isolate (0.4%, 1/231) (MIC 2 μg/mL).

1A-c. Comparison of in-house BMD and Etest results. Etest was performed on 127 isolates including 110 MRSA and 17 MSSA. By Etest, the MIC_{50s} of MRSA and MSSA were 0.75 and 0.5 μg/mL, while their MIC_{90s} were 1 and 0.5 μg/mL, respectively (Table 3). Comparisons of the MIC results obtained by in-house BMD and Etest are shown in Table 4. Among the 127 isolates, 49 (38.6%) had the same MIC results from both methods, while lower MIC was obtained in 42 (33.1%) and higher MIC was obtained in 36 (28.3%) isolates by Etest. Although the MIC result differences were within one 2-fold dilution, there were 4 daptomycin resistant MRSA isolates by Etest (3.6%, 4/110), albeit with MIC of 1.5 μg/mL. By BMD, 3 of these 4 isolates had MIC of 1 μg/mL and the other had MIC of 2 μg/mL.

1A-d. Correlation of daptomycin and vancomycin MIC levels. Figure 1 shows the daptomycin MIC distribution grouped by vancomycin MIC. Among the vancomycin MIC 1.0 μg/mL group (Van 1.0, n=216), 4.6%, 82.9%, and 12.5% had daptomycin MIC of 0.25, 0.5, and 1 μg/mL, respectively. In the Van MIC 2.0 μg/mL group (n=65), 61.5% had daptomycin MIC of 0.5 μg/mL, while 36.9% (24 isolates) had daptomycin MIC of 1.0 μg/mL, a rate significantly higher than the 12.5% ($p < 0.01$, χ^2 test) in the Van 1.0 group. The one daptomycin MIC 2.0 μg/mL isolate by BMD was from the Van 2.0 group, so were the other 3 isolates with daptomycin MIC 1.5 μg/mL by Etest.

IB. Daptomycin nonsusceptibility with heterogeneous Vancomycin intermediate resistance in MRSA. While performing studies on DAP-NS prevalence in *S. aureus*, we were contacted by Dr. Chen-Hsiang Lee of Chang Gung Memorial Hospital-Kaoshiung Medical Center on a patient with fatal persistent MRSA bacteremia. The MRSA developed daptomycin nonsusceptibility during high-dose daptomycin therapy. Prior to daptomycin, the patient received 4-weeks of teicoplanin followed by 5-months of oral linezolid. A total of 8 MRSA (CGK1 to CGK8) were recovered from the patient's blood cultures over 8 months of time (Table 5).

1B-A. Genotypes and MICs of isolates CGK 1-8. CGK1-4, CGK5-7, and CGK8 were from before, during, and after daptomycin therapy, respectively (see Table 3 for time course). All 8 isolates had indistinguishable pulsed-field gel electrophoresis pattern (data not shown). Genotypic of showed that all 8 isolates shared the same genetic profile. The isolates belonged to sequence type (ST) 59, and carried type IV *SCCmec*, and were

pvl-negative. These results indicated that the same strain of MRSA or its progeny was responsible for the persistent bacteremia is this patient.

The MICs of daptomycin and vancomycin were determined by BMD and Etest. Daptomycin MIC in CGK1-CGK5 were $\leq$ 0.75 μg/mL but increased to 4 μg/mL in CGK6-CGK8. CGK7 also developed nonsusceptibility to vancomycin (MIC 3 μg/mL), while the other isolates had MIC of 1-2 ug/mL (Table 5). Population analysis profile (PAP) performed for daptomycin showed a distinct increased population of CGK6-CGK8 able to grow in daptomycin concentrations of > 2 μg/mL (Fig 2A). PAP for vancomycin showed that CGK7 fit the hVISA criteria (Fig 2B).

Daptomycin nonsusceptibility in *S. aureus* is associated with mutations of genes affecting the cell membrane positive charges. Since mutations in the *mprF* gene, which encodes lysyl-phosphatidylglycerol (LPG) synthase and translocase, have been reported in several daptomycin resistant *S. aureus* strains, we also sequenced the *mprF* gene on all 8 isolates and found a single-point mutation (C to T) resulting in an amino acid substitution at position 431 from leucine to phenylalanine (L431F) in CGK6-CGK8 but no mutation was found in all 8 isolates in the sites (S295, P314, T345, I420) previously reported.

1C. Discussion on daptomycin and vancomycin non-susceptible *Staphylococcus aureus*. The majority of MRSA and MSSA in this study were susceptible to daptomycin. However, the MIC_{90} of the MRSA isolates at 1 μg/mL was 4-fold dilutions higher than the data reported by Sader et al., who found that among the 1800 MRSA bloodstream isolates from US hospitals, 92.9% had daptomycin MIC of $\leq$ 0.25 μg/mL [Sader et al., 2008]. The reason for the higher daptomycin MIC in our study may be due to the selection of a higher proportion of isolates with vancomycin MIC of 2.0 μg/mL. In the same study by Sader et al., it was also reported that higher daptomycin MIC results were obtained by Etest than by the reference BMD method. In our study, although the differences between BMD and Etest daptomycin MIC results were within 2-fold dilution, there were a few more isolates with resistant results by Etest.

Various mechanisms have been reported to be associated with daptomycin and vancomycin nonsusceptibility [Cui et al., 2006; Moise et al., 2009; Yang et al., 2009]. Although much remain to be elucidated, the causative strain in the present case with initial vancomycin MIC (2 μg/mL) at the upper susceptible limit with priming by glycopeptide exposure (teicoplanin in this case), and the lack of surgical drainage of infection foci, likely contributed to the development of nonsusceptibility and persistent MRSA

bacteremia in the present case. Development of practical laboratory methods for timely detection of strains with potential for emergence of heterogeneous resistance or attenuated susceptibility is needed. This study has been published in the September issue of AAC [Lee et al., 2010).

Part 2. MRSA Case Study

MRSA in Taiwan belong to 4 major clones based on PFGE pulsotypes. Isolates within the same pulsotype share very similar genetic background based on sequence type (ST), *SCCmec* type, presence of *pvl* toxin gene, and resistance profiles [Chen et al., 2005]. Pulsotype A isolates are ST239/241/SCC*mec* III/*pvl*-negative, pulsotype B isolates are ST59/SCC*mec* IV/*pvl*-negative, pulsotype C isolates are ST59/*SCCmec* IV/*pvl*-positive, while pulsotype D isolates are ST5/SCC*mec* II/*pvl*-negative. Pulsotype A isolates are mostly from inpatients, while pulsotype B and C are mostly from outpatients. Studies by researchers in Taiwan and our group have found that MRSA isolates belonging to pulsotype D have been increasing in Taiwan. Because of limited clinical information on MRSA isolates in the TSAR collection, a retrospective investigation was performed to investigate the disease spectrum, risk factors, infection status, and treatment outcome associated with these 4 major MRSA clones. A pre-designed case report form was used (attached). Because of the high correlation of SCC*mec* type with pulsotype, subsequent analysis was made by SCC*mec* type.

2A. MRSA cases. A total of 191 MRSA case reports from 4 hospitals were obtained. The 4 hospitals are located in the middle (1 with 71 cases, another with 34 cases), south (1 with 25 cases) and east (1 with 61 cases) regions. The cases were from 2002 (51 cases), 2004 (41 cases), 2006 (46 cases), and 2008 (53 cases). The case reports were completed and reviewed by each hospital infectious disease physician. Among these 191 MRSA cases, 149 had complete history available for analysis for this report. We are waiting for more complete data on the other 42 cases. Due to the late return of the questionnaires, preliminary analysis data on the 149 cases are presented here (Table 6). Among the 149 MRSA strains responsible for infections, 19 (12.8%), 51 (34.2%), 28 18.8%), and 51 (34.2%) were from MRSA strains carrying SCC*mec* II, SCC*mec* III, IV and V, respectively.

2B. Patient demographic. The age of the patients ranged from <1 to 91 yo, with a mean $\pm$ SD of 48.3 $\pm$ 27.3. Patients in the SCC*mec* V groups were significantly younger (mean $\pm$ SD, 29.5 $\pm$ 27.4) compared to patients in the other three SCC*mec* groups, which were: 62.7 $\pm$ 23.8, 56.4 $\pm$ 20.1, 48.9 $\pm$ 29.7, for SCC*mec* types II, III, IV, respectively. There were more pediatric patients in the SCC*mec* type IV (21.4%) and V (35.3%) compared to

SCC*mec* II (10.5%) and III (3.9%) groups. There were more male patients in each SCC*mec* group (range: 57.1% to 68.4%).

2C. Risk factors, underlying diseases and outcome (Table 6). The majority of the patients in the SCC*mec* II and III groups were inpatients, with only 3 (15.8%) and 9 (17.6%) patients, respectively presented as outpatients, of whom 1 and 6 of these patients were admitted. In contrast, 22 (43.1%) of the patients in the SCC*mec* V group presented as outpatients, and only 1 was admitted. Most patients in the SCC*mec* II (18, 94.7%) and III (43, 84.3%) groups had underlying diseases, whereas significantly fewer patients (15, 29.4%) in the SCC*mec* V group had underlying diseases ($p < 0.01$). Very few patients (2, 3.9%) in the SCC*mec* V group had prior invasive therapy compared to patients infected with SCC*mec* II (5, 26.3%) and III (11, 21.6%) MRSA ($p = 0.029$).

These MRSA caused a wide spectrum of infections, including skin and soft tissue infection (SSTI), surgical site infection, respiratory tract infection (RTI), bloodstream infection (BSI), osteomyelitis, and other infections. However, more patients in the SCC*mec* II group had RTI (11, 57.9%) and BSI (10, 52.6%), whereas SSTI (19, 37.3%) and BSI (15, 29.4%) were the most common infections in the SCC*mec* III group, while BSI (10, 35.7%) and RTI (8, 28.6%) were most common in the SCC*mec* IV group. In contrast, the majority of the infections caused by SCC*mec* V isolates were SSTI (34, 66.7%). The majority of the patients recovered (cured or improved) from the MRSA infections, ranging from 52.6% in the SCC*mec* II group to 88.2% in the SCCmec V group. Direct and indirect attributable death was 26.3% (5 patients), 11.0% (6 patients), 3.6% (1 patient), and 5.9% (3 patients), while direct attributable death was 21.1% (4 patients), 3.9% (2 patients), 3.6% (1 patient), and 3.9% (2 patients) in the SCC*mec* II, III, IV, and V groups, respectively ($p = 0.079$).

2D. Discussion on MRSA case study. Although more cases and multivariate analysis are needed, this preliminary analysis indicated that significant differences exist in the patients infected by the 4 major MRSA clones in Taiwan. SCC*mec* V has been considered community-MRSA, and our data indicated that patients in this group are younger and fewer of them had underlying diseases and other risk factors. The overall mortality attributable to MRSA infections in these 149 patients was 6.0% (9 patients). The mortality rate could be higher since some patients (7 patients) were discharged against advices (AAD). In addition, patients in the SCC*mec* II group had the highest mortality (21.1%), which is a cause for concern since previous TSAR data and reports from other study groups in Taiwan have indicated increased SCC*mec* II MRSA infections in Taiwan hospitals.

Part 3. Carbapenem resistant (CR) *E. coli* and *K. pneumoniae* study.

E. coli and *K. pneumoniae* are the most common species of *Enterobacteriaceae* causing both community and hospital acquired infections. Increased resistance to extended spectrum β-lactams, such as 3rd and 4th generation cephalosporins in *E. coli* and *K. pneumoniae* have necessitated the use of carbapenems for treating infections caused by these resistant bacteria. Based on data from the last round of TSAR conducted in 2008, carbapenem resistance in *E. coli* and *K. pneumoniae* remained low (<1%). However, the sharp increase of carbapenem resistance in *Acinetobacter baumannii* in Taiwan in the last few years (from <3% overall in 2002 to 49.5% in 2008) serves as a reminder the importance of timely and accurate detection of carbapenem resistance in enteric pathogens.

3A. Isolates & patients. Because routine collection for the next round of TSAR was not scheduled to start until July 2010, we conducted a special TSAR collection for *E. coli* and *K. pneumoniae* isolates suspected to be carbapenem (ertapenem, imipenem, or meropenem) resistant. Six TSAR hospitals were invited but only 3 hospitals submitted isolates. A total of 71 isolates received from 3 hospitals were carbapenem resistant, including 23 *E. coli* and 48 *K. pneumoniae*. The majority of the isolates were from one hospital. These isolates were recovered between September 2009 and June 2010. Several patients had isolates recovered from multiple body sites and/or on different days. Thus the 71 isolates were from 53 patients with an average age of 71 yo (median 76 yo), indicating that elderly patients are the high risk group. Some of these CR *E. coli* and *K. pneumoniae* isolates were resistant to ertapenem only, while some were resistant to all ertapenem, imipenem, and meropenem (Tables 7 and 8).

3B. Detections of ESBL and plasmid-mediated AmpC β-lactamase genes. The results of phenotypic and genotypic detection of for genes encoding ESBL, plasmid-mediated AmpC β-lactamase (*bla*$_{\text{ESBL}}$ and *bla*$_{\text{AmpC}}$) on these CR *E. coli* and *K. pneumoniae* are shown in Tables 7 and 8, respectively. Among the 23 *E. coli* isolates studied, CTX-M-type *bla*$_{\text{ESBL}}$ were detected in 5 (21.8%) isolates from 4 patients, and *bla*$_{\text{AmpC}}$ were detected in 21 (91.3%) isolates (from 16 patients) including 1 DHA-type and 20 CMY-type. The 5 isolates positive for CTX-M were also positive for *bla*$_{\text{AmpC}}$ (1 DHA-type and 4 CMY-type). In the 48 *K. pneumoniae* isolates, 4 were positive for genes encoding SHV-type *bla*$_{\text{ESBL}}$, 25 were positive for CTX-M type *bla*$_{\text{ESBL}}$. In contrast to E. coli, which carried mostly CMY-type *bla*$_{\text{AmpC}}$, there were more DHA (23 isolates) than CMY (12 isolates) -type *bla*$_{\text{AmpC}}$ in *K. pneumoniae*. In *K. pneumoniae,* 19 isolates carried both *bla*$_{\text{ESBL}}$ (18 CTX-M type and 1 SHV-type) (Table 9). Three isolates from the same

patient carried both CTX-M bla_{ESBL} and carbapenemase bla_{IMP-8}.

3C. Carbapenemase detection. No carbapenemase was detected in the 23 CR-*E. coli* isolates. In *K. pneumoniae*, 3 isolates were positive for class B carbapenemase IMP-8, all 3 were all from the same patient, 2 of which were from bile and blood on the same day and the other was from sputum cultured 13 days later. The 3 IMP-8 isolates were also positive for CTX-M. The MIC of ertapenem was > 32 μg/mL for all 3 isolates. Imipenem MICs were 12, >32, >32 μg/mL for the 1st, and 2nd, 3rd isolate, respectively, while meropenem MIC remained at 6 μg/mL. PCR for the newly emerged bla_{NDM-1} (New Delhi metallo-β-lactamase gene) was also performed on all 71 isolates and none was detected.

3D. Clonal relatedness of carbapenem-resistant *E. coli* and *K. pneumoniae*. PFGE was performed to look for clonal relatedness and inter- or intra-hospital spread of CR-NS *E. coli* and *K. pneumoniae* (Fig. 3 and 4). In *E. coli*, 4 isolates from 3 patients isolated a month apart shared indistinguishable PFGE pattern (Fig 3). In *K. pneumoniae*, 3 clusters (12 isolates from 9 patients in cluster A, 11 isolates from 7 patients in cluster B, 3 isolates from 3 patients in cluster C) of isolates from 1 hospital showed >80% similarity in PFGE pattern. These isolates were either from the same patient recovered from different body sites, from the same specimen type on different day, or from different patients and isolates with indistinguishable pattern were found within each cluster. The 3 IMP-8 positive isolates from the same patient had identical PFGE pattern (Fig 4).

3E. Discussions on carbapenem non-susceptible *E. coli* and *K. pneumoniae* study. The emergence of carbapenem-resistant *E. coli* and *K. pneumoniae* is a major cause for concern since carbapenems are the drugs of choice for treating Gram-negative enteric bacteria resistant to extended spectrum cephalosporins. To the best of our knowledge, *K. pneumoniae* carrying the genes encoding MBL IMP-8 has rarely been reported in Taiwan except in one medical center [Yan et al., 2008]. Although further studies are needed, in isolates negative for carbapenemase genes, carriage of one or multiple β-lactamases genes (5 *E. coli*, and 19 *K. pneumoniae*) plus loss of outer membrane protein likely contributed to their carbapenemase resistance. The finding of multiple isolates with identical or highly similar PFGE patterns from different patients indicated that clonal spread has occurred. Although the majority of the CR-*E. coli* and CR-*K. pneumoniae* isolates were from one hospital, they were also found in 2 other hospitals. Since some of these CR isolates were resistant to ertapenem only, and not all hospitals use ertapenem to screen for carbapenem-resistance, *E. coli* and *K. pneumoniae* with low level carbapenem resistance may be missed in those hospitals.

Part 4. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) study

The prevalence of CRAB has increased dramatically in the last few years in Taiwan. The CRAB are of great concern because they are nearly all resistant to other treatment choices for *A. baumannii*, such as amikacin, ceftazidime, levofloxacin. Based on TSAR data, CRAB comprised <3% in 2002, but increased to 16% in 2004, 32% in 2006, and 49% in 2008 ($p < 0.001$). In addition, as high as 66% of *A. baumannii* from ICU patients in TSAR VI (2008) were CRAB, compared to 15% in 2004 and 45% in 2006. Differences in CRAB rates were noted in isolates from different TSAR hospitals and regions. Since TSAR isolates were collected over a three months (July to September) period only, and some hospitals had few isolates so was difficult for inter-hospital comparison, plus collection for the 7th round of TSAR was not scheduled to take place until July 2010, we conducted a special TSAR collection for all *A. baumannii* from 6 TSAR hospitals. This study was performed to determine the overall rate of CRAB in ICUs of different hospitals and to see if there is a predominant clone of CRAB in each hospital or in all hospitals, and if there are inter-hospital and intra-hospital spread.

4A. Isolates. Between September 2009 and March 2010, a total of 587 isolates were collected from ICU of 6 hospitals. These isolates came from 421 patients. Between 23 and 170 isolates from 19 to 120 patients were collected from the 6 hospitals. The majority of the isolates were from 3 hospitals in the middle region (range: 94 - 170 isolates), while the hospital in the north, south and east contributed 165, 23, and 26 isolates, respectively.

4B. Prevalence of carbapenem resistance in *A. baumannii* from ICU. Due to the large number of isolates, antimicrobial susceptibility was performed by disk diffusion to screen for CRAB. Carbapenem resistance (as defined by resistance to imipenem and/or meropenem) was 79.2% overall. Rates of carbapenem resistance were high in isolates from all specimen types but were highest in isolates from respiratory tracts (82.2%) and pus/wound (81.6%), compared to those from blood (66.7%), urine (58.3%) and other specimen types (66.7%). The rates of CRAB among isolates from the 6 hospitals were 73.9% (17/23), 83.6% (140/165), 84.6% (22/26), 99.1% (108/109), 57.1% (97/170), and 88.3% (83/94) for hospitals A to F, respectively. Since some patients had multiple isolates, we also calculated the CRAB rates on only the first isolate of each patient to determine the effect of multiple isolates on resistance rate. When only the first isolate of each patient was included for analysis, CARB rate in hospital B changed from 83.6% (140/165) to 74.7% (59/79) (Fig 5). The rates of CRAB did not differ in the other 5 hospitals when only the first isolates were analyzed.

4C. Screening of CRAB for *bla*$_{\text{NDM-1}}$. The mechanisms of carbapenem resistance in CRAB have been well studied by different research groups. For this project last year, we had already analyzed CRAB from previous rounds of TSAR and showed the association of IS*Aba*1 with the *bla*$_{\text{oxa.}}$carbapenemases OXA-23 and OXA-51 in CRAB [Segar et al., 2007; Turton et al., 2006]. Therefore this year we only looked for *bla*$_{\text{NDM-1}}$ on all 472 CRAB but did not detect any positive ones.

4D. Clonal relatedness of CRAB. Because of the large numbers of CRAB, we randomly selected a total of 108 CRAB from these hospitals to perform PFGE to determine if a predominant clone exists in each hospital of in different hospitals. The dendrogram of CRAB from each hospital is presented in Fig. 6A to 6F. Although isolates from each hospital are distinct from isolates from other hospitals (data not shown), there were several pairs and clusters of isolates from different patients within each hospital having indistinguishable or closely related PFGE patterns, including some isolates recovered more than 1 month apart and even at more than 6 months apart (ex. A008 and 015 pair; C006-008, C010-11, C013, C022 cluster; D066, D073, D091 cluster; F052 and F060 pair). The same CRAB strain could also be present on multiple body sites simultaneously (patient EH) and from different times even 6 weeks apart (patient EF).

4E. Discussion on CRAB study. Data from this study indicated that CRAB rates differed significantly among *A. baumannii* isolated from ICU patients in different hospitals in Taiwan (57.1% to 99.1% overall, and 56.7% vs. 99% on first isolates). Our analysis also shows that it is important to standardize analysis of resistance data if comparisons among hospitals are to be made since multiple isolates from the same patient can affect resistance rates. We also showed that considerable heterogeneity exist in CRAB strains circulating in different hospitals. However, we also found multiple closely related or indistinguishable CRAB strains within each hospital, thus some transmission of CRAB among patients within each hospital likely occurred. Our data also showed that the same CRAB strain can persist on the same patient for several weeks. Of noteworthy also is that *A. baumannii* from 5 of the 6 hospitals ICU surveyed had CRAB rates >70% including one as high as 99%. Taken together these data indicate the urgent need for more stringent infection control measures.

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

The majority of MRSA in Taiwan remained susceptible to daptomycin (DAP). However, daptomycin and vancomycin nonsusceptible MRSA can emerge to result in treatment failure during therapy. Preliminary analysis of the MRSA cases indicated that significant differences exist in the patients infected by the 4 major MRSA clones in Taiwan. SCC*mec* V has been considered community-MRSA, and our data indicated that patients in this group are younger and fewer of them had underlying diseases and other risk factors. The overall mortality attributable to MRSA infections in these 149 patients was 6.0% (9 patients) but patients in the SCC*mec* II group had higher mortality (21.1%). Because of the small sample size in SCC*mec* II group, more cases and multivariate analysis are needed to confirm these findings.

The finding of multiple carbapenem-resistant *E. coli* and *K. pneumoniae* isolates with identical or highly similar PFGE patterns from different patients indicated that clonal spread has occurred. Since some of these CR isolates were resistant to ertapenem only, and since not all hospitals use ertapenem to screen for carbapenem-resistance, *E. coli* and *K. pneumoniae* with low level carbapenem resistance may be missed in some hospitals. The majority of the patients were elderly (>70 yo). The prevalence of CRAB has increased dramatically in the last few years in Taiwan. TSAR VI (2008) data indicated that 66% of *A. baumannii* from ICU patients of 26 hospitals were CRAB, compared to 15% in 2004 and 45% in 2006. The present study found rates of CRAB from ICUs of 6 hospitals to be 78.1% overall but considerable differences in CRAB rates existed between the 6 hospitals. Clonal spread of CRAB within each hospital also occurred.

We conclude that careful monitoring of emergence of reduced susceptibility to daptomycin and vancomycin in patients with severe MRSA infections who are being treated by either of these two agents. We also suggest that active surveillance for carbapenem resistance in *Enterobacteriaceae* and *A. baumannii* in high risk patient groups, such as those in ICU, is warranted. Enforcement of stringent infection control measures in high risk areas of the hospitals is urgently needed.

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(七) 圖表 Tables and Figures.

Table 1. Primers used for detection of beta-lactamase genes encoding ESBL and AmpC.

Enzyme family	Primer name	Primer sequence (5`-3` direction)	Nucleotide positions (in GenBank)	Fragment size (bp)	Reference
Extended spectrum β-lactamase (ESBL)					
SHV	bla-SHV.SE	ATGCGTTATATTCGCCTGTG	1-21	747	Monsterin et al., 2007
	bla-SHV.AS	TGCTTTGTTATTCGGGCCAA	753-734		
TEM	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	140863-140837	445	Monsterin et al., 2007
	TEM-164.AS	ACGCTCACCGGCTCCAGATTTAT	140419-140441		
CTX-M	CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC		593	Monsterin et al., 2007
	CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG[a]			
AmpC beta-lactamases					
CMY-1, 8 to 11	MOXMF	GCTGCTCAAGGAGCACAGGAT	358–378	520	Perez-Perez et al., 2002
	MOXMR	CACATTGACATAGGTGTGGTGC	877–856		
CMY-2 to 7	CITMF	TGGCCAGAACTGACAGGCAAA	478–498	462	Perez-Perez et al., 2002
	CITMR	TTTCTCCTGAACGTGGCTGGC	939–919		
DHA-1 & DHA-2	DHAMF	AACTTTCACAGGTGTGCTGGG T	1244–1265	405	Perez-Perez et al., 2002
	DHAMR	CCGTACGCATACTGGCTTTGC	1648–1628		

a. R stands for purine, Ystands for pyrimidine, and S stands for G or C.

Table 2. Primers used for detection of genes encoding carbapenemase

Enzyme family	Primer	Primer sequence (5'-3')	Fragment size (bp)	Reference
Class A carbapenemases				
NMC	NMC1*	GCATTGATATACCTTTAGCAGAGA	2,158	Queenan & Bush, 2007
	NMC4	CGGTGATAAAATCACACTGAGCATA		
SME	IRS-5	AGATAGTAAATTTTATAG	1,138	Queenan & Bush, 2007
	IRS-6	CTCTAACGCTAATAG		
IMI	IMI-A	ATAGCCATCCTTGTTTAGCTC	818	Queenan & Bush, 2007
	IMI-B	TCTGCGATTACTTTATCCTC		
KPC	KPC-F	ATGTCACTGTATCGCCGTCT	893	Queenan & Bush, 2007
	KPC-R	TTTTCAGAGCCTTACTGCCC		
GES	GES-C	GTTTTGCAATGTGCTCAACG	371	Queenan & Bush, 2007
	GES-D	TGCCATAGCAATAGGCGTAG		
Class B metalloenzymes				
IMP	IMP-F	GGAATAGAGTGGCTTAATTCTC	188	Ellington et al, 2007
	IMP-R2	CCAAACYACTASGTTATCT		
VIM	VIM-F	GATGGTGTTTGGTCGCATA	390	Ellington et al., 2007
	VIM-R	CGAATGCGCAGCACCAG		
GIM	GIM-F	TCGACACACCTTGGTCTGAA	477	Ellington et al., 2007
	GIM-R	AACTTCCAACTTTGCCATGC		
SPM	SPM-F	AAA ATCTGG GTA CGC AAA CG	271	Ellington et al., 2007
	SPM-R	ACATTATCCGCTGGAACAGG		
NDM-1	NDM-1-F	ATGCGGGCCGTATGAGTGA	678	Limbago et al., 2010
	NDM-1-R	GGAAACTGGCGACCAACG		
NDM-1	NDM-2-F	CTCATGTTTGAATTCGCCC	1014	Limbago et al., 2010
	NDM-2-R	ACTCGTCGCAAAGCCCAG		
Class D oxacillinases				
Subgroup 6 (OXA-48)	OXA-48A	TTGGTGGCATCGATTATCGG	744	Queenan & Bush, 2007
	OXA-48B	GAGCACTTCTTTTGTGATGGC		

Table 3. In vitro activity of daptomycin against methicillin -resistant and -susceptible *Staphylococcus aureus* (MRSA & MSSA)

Test method (no. of isolates)	MIC (μg/mL)			Susceptibility[a]	
	Range	MIC_{50}	MIC_{90}	%S	%NS
Broth microdilution (In-house)					
MRSA (231)	0.25-2	0.5	1	99.6	0.4
MSSA (61)	0.12-1	0.5	0.5	100	0
All (292)	0.12-2	0.5	1	99.7	0.3
Etest					
MRSA (110)	0.25-1.5	0.75	1	96.4	3.6
MSSA (17)	0.12-0.75	0.5	0.5	100	0
All (127)	0.12-1.5	0.5	1	96.9	3.1

[a] S, susceptible (MIC ≤ 1 μg/mL); NS, resistant (MIC > 1μg/mL).

Table 4. Comparison of daptomycin MIC results by in-house broth microdilution test (BMD) and Etest

Daptomycin MIC (μg/mL)		No. of isolates
BMD	Etest	
0.12	0.12	1
0.25	0.38	3
0.5	0.25	3
	0.38	22
	0.5	32
	0.75	25
	1.0	5
1.0	0.38	1
	0.5	2
	0.75	13
	1.0	16
	1.5	3
2.0	1.5	1

Table 5. Minimal inhibitory concentrations (MICs) of daptomycin and vancomycin in 8 MRSA isolated from a fatal persistent bacteremia case

Isolate	Isolation date	Day of daptomycin therapy[a]	MIC (μg/mL)[b]				
			Daptomycin	Linezolid	Oxacillin	Teicoplanin	Vancomyci
CGK1	12/1/08	-	0.5	2	32	1	1.5
CGK2	1/16/09	-	0.75	2	>256	1.5	1.5
CGK3	4/16/09	-	0.75	2	>256	1.5	2
CGK4	6/22/09	-	0.38	2	16	1	1.5
CGK5	7/6/09	11	0.75	2	>256	2	2
CGK6	7/12/09	17	4	1	1.5	2	2
CGK7	7/21/09	26	4	2	3	3	3
CGK8	7/27/09	-	4	1.5	1	2	2

[a] The patient was on teicoplanin (12/2/08 to 1/5/09), oral linezolid (1/17/09 to 4/14/09 and 4/16/09 to 6/22/09), daptomycin (6/25/09 to 7/21/09), and linezolid plus cefpirome (7/24/09 to 7/28/09)

[b] MICs results are from Etest (AB Biodisk, Solna, Sweden).

Table 6. The demographic and clinical data of 149 patients with MRSA infections

Parameters	MRSA group based on SCC*mec* type				*p* value
	SCC*mec* II	SCC*mec* III	SCC*mec* IV	SCC*mec* V	
	n =19	n = 51	n = 28	n+ 51	
Age, mean ± SD (yo)	62.7 ± 23.8	56.4 ± 20.1	48.9 ± 29.7	29.5 ± 27.4	<0.01
Age group, N (%)					<0.01
Ped (<=15 yo)	2 (10.5)	2 (3.9)	6 (21.4)	18 (35.3)	
Adu (16-64 yo)	5 (26.3)	20 (28.8)	14 (50.0)	25 (49.0)	
Elderly (>= 65 yo)	12 (63.2)	19 (37.3)	8 (28.6)	8 (15.7)	
Gender, N (%)					0.909
Male	13 (68.4)	31 (60.8)	16 (47.1)	32 (62.7)	
Location, N (%)					0.039
ICU	6 (31.6)	15 (29.4)	6	5 (9.8)	
Non-ICU inpatients	10 (52.6)	27 (52.9)	15	24 (47.1)	
Outpatients (OPD)	3 (15.8)	9 (17.6)	7	22 (43.1)	
Admitted, N (% of OPD)	1 (33.3)	6 (66.7)	1 (14.3)	1 (14.5)	
Specimen, N (%)					
Abscess/pus/wound	4 (21.1)	19 (37.3)	13 (46.4)	41 (80.4)	
Blood	6 (31.6)	14 (27.5)	7 (25.0)	4 (7.8)	
Respiratory	3 (15.8)	3 (5.9)	3 (10.7)	1 (2.0)	
Tip	2 (10.5)	8 (15.7)	1 (3.6)	0	
Urine	2 (10.5)	3 (5.9)	1 (3.6)	1 (2.0)	
Other	2 (10.5)	4 (7.8)	3 (10.7)	4 (7.8)	
LOS (days), mean ± SD	52.7 ± 82.3	51.9 ± 96.8	17.4 ± 24.0	7.6 ± 13.1	0.003
Underlying diseases, N (%)	18 (94.7)	43 (84.3)	19 (67.9)	15 (29.4)	<0.01
Prior invasive therpy, N (%)					0.029
Yes	5 (26.3)	11 (21.6)	4 (14.3)	2 (3.9)	
No	14 (73.7)	40 (78.4)	24 (85.7)	48 (94.1)	
Unknown	0	0	0	1 (2.0)	
Discharge diagnosis, N (%)					0.139
Infection due to MRSA, N (%)	18 (94.7)	45 (88.2)	27 (96.4)	49 (96.1)	
Skin and soft tissue	7 (36.8)	19 (37.3)	6 (21.4)	34 (66.7)	<0.01
Surgical site	2 (10.5)	5 (9.8)	4 (14.3)	3 (5.9)	0.641
Respiratory tract	11 (57.9)	11 (21.6)	8 (28.6)	3 (5.9)	<0.01
Urinary tract	6 (31.6)	8 (15.7)	0	1 (2.0)	<0.01
Blood stream	10 (52.6)	15 (29.4)	10 (35.7)	2 (3.9)	<0.01
Ear	1 (5.3)	1 (2.0)	1 (3.6)	2 (3.9)	0.92
Gastrointestinal	0	1 (2.0)	1 (3.6)	1 (2.0)	1
Osteomyolitis	1 (5.3)	4 (7.8)	3 (10.7)	1 (2.0)	0.338
Other systems	0	6 (11.8)	2 (7.1)	4 (7.8)	0.554
Outcome, N (%)					
AAD	1 (5.3)	1 (2.0)	4 (14.3)	1 (2.0)	
Cured/improved	10 (52.6)	35 (68.6)	21 (75.0)	45 (88.2)	
Death (indirect & direct)	5 (26.3)	6 (11.0)	1 (3.6)	3 (5.9)	
Death due to this MRSA	4 (21.1)	2 (3.9)	1 (3.6)	2 (3.9)	0.079
No follow up	0	3 (5.9)	1 (3.6)	2 (3.9)	
Transferred	3 (15.8)	6 (11.8)	1 (3.6)	0	

LOS, length of stay

Table 7. Phenotypic and genotypic detection results of 23 carbapenem resistant *E. coli*

MIRLNO	Patient	Specimen	CDATE	HAI	MHT-Ertapenem	Carbapenem-R	ESBL confirmatory test	*bla* ESBL	*bla* AmpC	Carbapenemase
B207		Sputum	2009/10/08		Neg	EI	Pos	CTX-M	DHA-type	neg
D218	O	Bile	2010/1/29		Neg	EI	Neg		CMY-type	neg
D219	O	Ascites	2010/2/1		Neg	EIM	Neg		CMY-type	neg
E202	P	Bile	2009/09/04		Neg	EIM	Pos	CTX-M	CMY-type	neg
E208		Bile	2009/09/19		Pos	E	Pos	CTX-M	CMY-type	neg
E210	P	Bile	2009/09/21		Neg	EIM	Pos	CTX-M	CMY-type	neg
E211		Discharge	2009/09/26		Neg	EIM	Neg	CTX-M	CMY-type	neg
E214	E	Blood	2009/10/03	Y	Neg	EIM	Neg		CMY-type	neg
E215	E	Blood	2009/10/02	Y	Neg	EIM	Neg		CMY-type	neg
E217		Urine	2009/10/08		Pos	E	Neg		CMY-type	neg
E225		Urine	2009/10/23		Pos	EIM	Neg		CMY-type	neg
E232	L	Discharge	2009/12/13		Neg	E	Neg			neg
E233	L	Discharge	2009/12/14		Pos	E	Neg			neg
E238	L	Discharge	2009/12/23		Neg	E	Neg		CMY-type	neg
E249		Drainage	2010/01/22		Pos	EI	Neg		CMY-type	neg
E285	S	Discharge	2010/03/12		Neg	E	Neg		CMY-type	neg
E508	S	Sputum	2010/04/09		Neg	E	Neg		CMY-type	neg
E513		Bile	2010/04/16		Neg	E	Neg		CMY-type	neg
E526		Urine	2010/05/03		Neg	E	Neg		CMY-type	neg
E537		Discharge	2010/05/15		Neg	EIM	Neg		CMY-type	neg
E547	T	Drainage	2010/06/06		Neg	EI	Neg		CMY-type	neg
E554		Drainage	2010/06/18		Neg	EIM	Neg		CMY-type	neg

MHT, modified Hodge Test.

Carbapenem-R (including intermediates), E, ertapenem; I, imipenem; M, meropenem.

Table 8. Phenotypic and genotypic detection results of 49 carbapenem resistant *K. pneumoniae*

MIRLNO	Patient	Specimen	CDATE	HAI	MHT-Ertapenem	Carbapenem-R	ESBL confirmatory	*bla* ESBL	*bla* AmpC	Carbapenemase
B209		Urine	2009/10/04		Neg	E	Pos	SHV		neg
B229	R	Sputum	2009/11/26		Neg	EIM	Pos	CTX-M	DHA-type	neg
B231	R	Sputum	2009/11/25		Neg	EIM	Pos	CTX-M	DHA-type	neg
B295		Sputum	2010/03/30		Neg	EM	Pos	SHV		neg
D208		Urine	2009/11/5	V	Neg	EI	Pos	SHV	DHA-type	neg
D215		Tip	2010/1/8		Pos	EI	Neg		DHA-type	neg
D217		Bile	2010/1/30		Pos	EIM	Neg			neg
D221		Urine	2010/2/12		Pos	EI	Neg	CTX-M	DHA-type	neg
D227		Urine	2010/3/17		Pos	EIM	Neg			neg
D228		Urine	2010/3/17		Neg	EIM	Neg			neg
E204	D	Bile	2009/09/06		Pos	EIM	Pos	CTX-M		IMP-8
E205	D	Blood	2009/09/06		Pos	EIM	Pos	CTX-M		IMP-8
E207		Blood	2009/09/16		Neg	EI	Pos	CTX-M	DHA-type	neg
E209	D	Sputum	2009/09/19		Pos	EIM	Neg	CTX-M		IMP-8
E213		Urine	2009/09/30		Neg	E	Pos	CTX-M	DHA-type	neg
E216	J	Tip	2009/10/06	Y	Neg	E	Neg		CMY-type	neg
E219	I	Sputum	2009/10/14		Neg	E	Pos	CTX-M	DHA-type	neg
E220	I	Sputum	2009/10/15		Neg	E	Pos	CTX-M	DHA-type	neg
E222		Urine	2009/10/19		Neg	EI	Neg		CMY-type	neg
E223		Urine	2009/10/19		Neg	EI	Pos	CTX-M	DHA-type	neg
E224	K	Liver	2009/10/23		Neg	EIM	Pos	SHV		neg
E227		Pus/Wound	2009/11/03		Neg	E	Neg		CMY-type	neg
E229		Urine	2009/12/03		Neg	E	Neg		CMY-type	neg
E230		Sputum	2009/12/03		Neg	EI	Neg		CMY- & DHA-type	neg
E231	K	Liver	2009/12/10		Neg	EIM	Pos	CTX-M	DHA-type	neg
E235		Sputum	2009/12/15		Neg	EIM	Neg	CTX-M	DHA-type	neg
E236		Urine	2009/12/21		Neg	EIM	Neg			neg
E239	M	Urine	2009/12/30		Neg	EIM	Neg		CMY-type	neg
E240	M	Urine	2010/01/01		Neg	EIM	Neg		CMY-type	neg
E241	M	Sputum	2009/12/31		Neg	EIM	Neg		CMY-type	neg
E243		Sputum	2010/01/17		Neg	EI	Neg	CTX-M	DHA-type	neg
E244		Urine	2010/01/05		Neg	E	Neg	CTX-M		neg
E248		Pus/Wound	2010/01/20		Neg	EI	Pos	CTX-M	DHA-type	neg
E250	N	Bile	2010/02/02		Neg	E	Pos	CTX-M	DHA-type	neg
E283	U	Bile	2010/03/08		Neg	EIM	Neg	CTX-M		neg
E284	V	Urine	2010/03/11		Pos	EIM	Neg		CMY-type	neg
E286	N	Bile	2010/03/16		Neg	EIM	Neg	CTX-M	DHA-type	neg
E288		Bile	2010/03/27		Neg	EIM	Neg	CTX-M	DHA-type	neg
E501		Blood	2010/03/31		Neg	EIM	Neg		CMY-type	neg
E502		Blood	2010/03/31		Neg	E	Pos	CTX-M	DHA-type	neg
E507	U	Bile	2010/04/09		Neg	E	Neg	CTX-M		neg
E510	V	Sputum	2010/04/15		Neg	EIM	Neg		CMY-type	neg
E511	N	Drainage	2010/04/15		Neg	E	Pos	CTX-M	DHA-type	neg
E525		Urine	2010/05/03		Neg	E	Neg		DHA-type	neg
E527		Drainage	2010/05/06		Neg	E	Neg	CTX-M		neg
E540		Sputum	2010/05/18		Neg	EIM	Neg		CMY-type	neg
E553		Sputum	2010/06/16		Neg	E	Neg		DHA-type	neg
E555		Bile	2010/06/19		Neg	EIM	Neg	CTX-M	DHA-type	neg

Table 9. Summary of the presence of β-lactamase genes (*bla)* in carbapenem-resistant *E. coli* and *K. pneumoniae*

Species	*bla*			No of isolates
	ESBL	AmpC	Carbapenemase	(No. patients)
E. coli (23 isolates from 16 patients):				
	CTX-M	DHA	Neg	1 (1)
	CTX-M	CMY	Neg	4 (3)
	Neg	CMY	Neg	16 (12)
	Neg	Neg	Neg	2 (1)
K. pneumoniae (48 isolates from 37 patients):				
	SHV	Neg	Neg	3 (3)*
	SHV	DHA	Neg	1 (1)
	Neg	CMY	Neg	11 (8)
	Neg	DHA	Neg	3 (3)
	Neg	CMY & DHA	Neg	1 (1)
	CTX-M	DHA	Neg	18 (14)
	CTX-M	Neg	Neg	4 (3)
	CTX-M	Neg	IMP	3 (1)
	Neg	Neg	Neg	4 (4)

*One of the patients had a different isolate positive for $bla_{\text{CTX-M and DHA}}$.

Figure 1. Distribution of daptomycin MIC (Dap 0.12-2.0 μg/mL) grouped by vancomycin MIC (Van ≤ 0.5, 1.0, 2.0 μg/mL) in 292 *S. aureus*

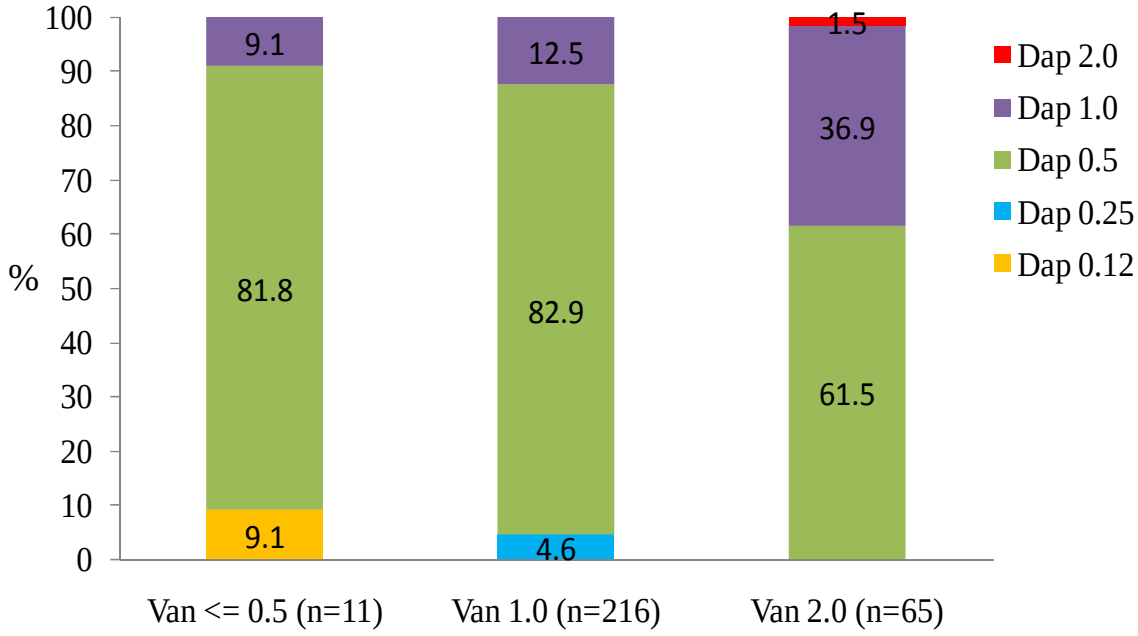
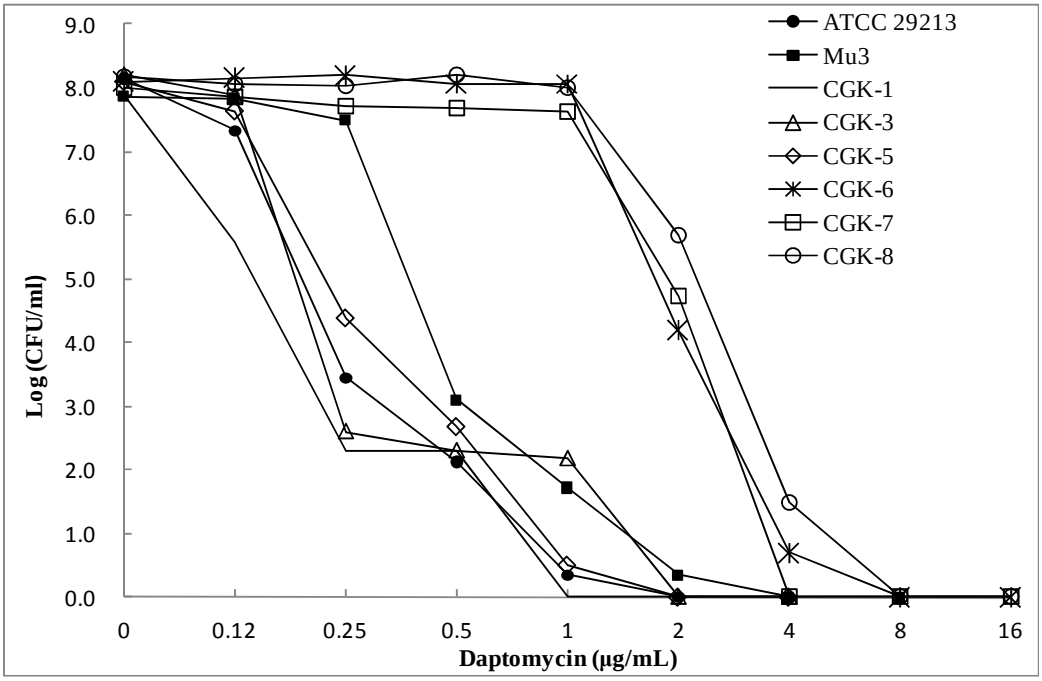


Figure 2. Population analysis profiles (PAP) of daptomycin (A) and vancomycin (B) on 6 of 8 sequential MRSA isolates from a fatal persistent bacteremia case. CGK1, CGK3, and CGK5 were daptomycin susceptible and CGK6-CGK8 isolates were daptomycin resistant. N315, hVISA negative control; Mu3, hVISA positive control.

2A.



2B.

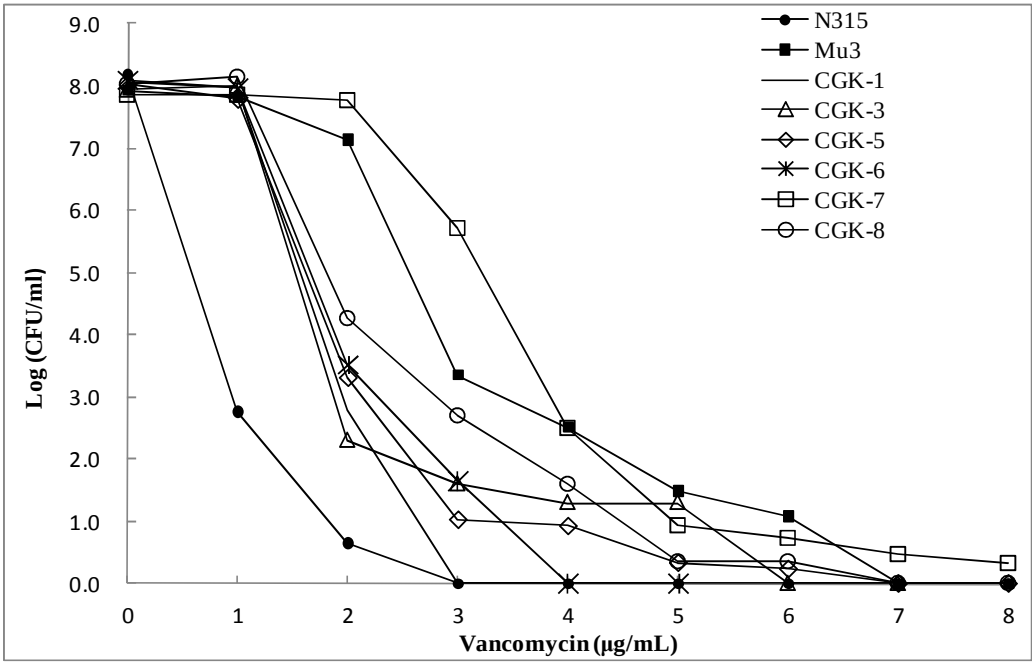
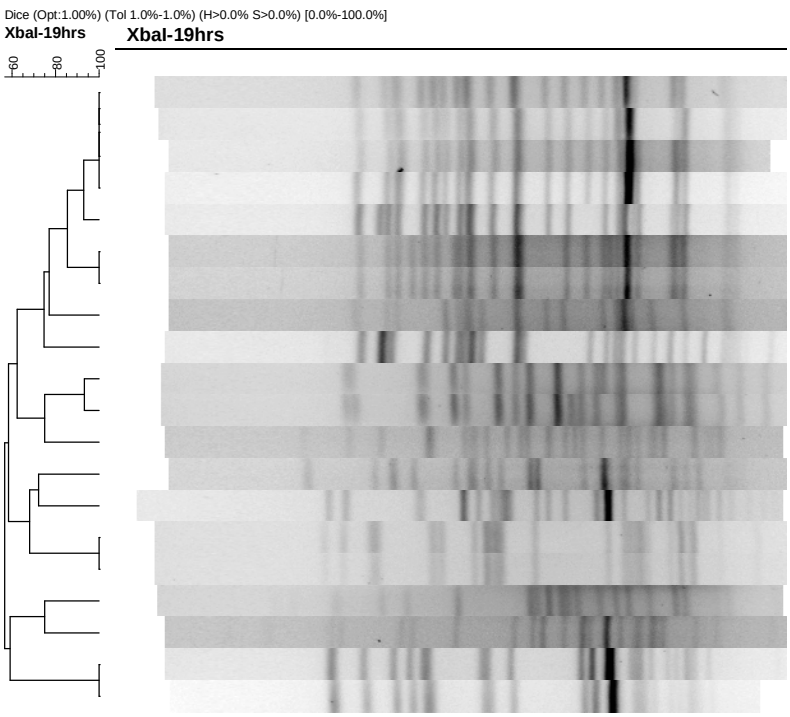
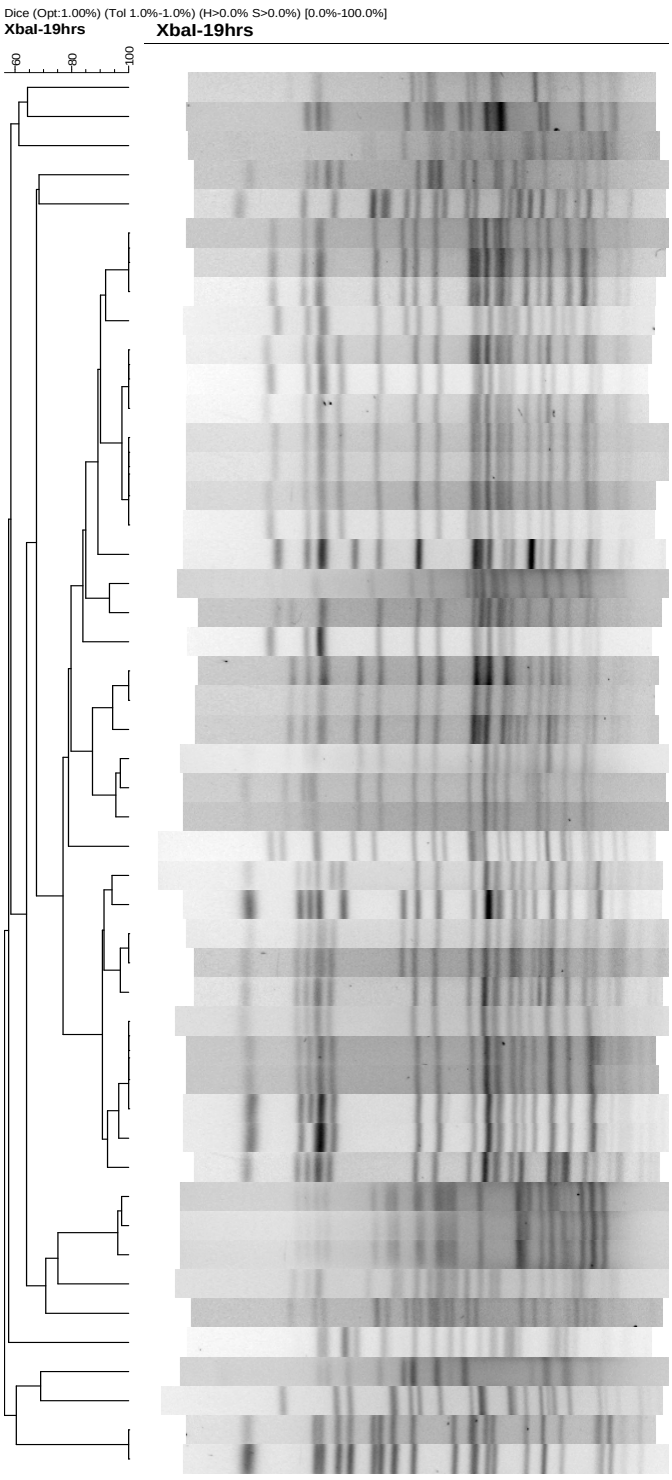


Figure 3. Dendrogram of *Xba*I digested chromosomal DNA of 23 carbapenem resistant *E. coli*, with culture date (CDATE), specimen type, and β-lactamase (*bla*) ESBL (SHV and CTX-M), AmpC, and carbapenemase. Letters under Patient indicate isolates from the same patient.



MIRLNO*	Patient	CDTATE	Specimen	SHV	CTX-M	AmpC	Carbapenemase
E238	L	2009/12/23	Discharge	-	-	CMY	-
E249		2010/01/22	Drainage	-	-	CMY	-
E285	S	2010/03/12	Discharge	-	-	CMY	-
E508	S	2010/04/09	Sputum	-	-	CMY	-
E526		2010/05/03	Urine	-	-	CMY	-
E214	E	2009/10/03	Blood	-	-	CMY	-
E215	E	2009/10/02	Blood	-	-	CMY	-
E211		2009/09/26	Discharge	-	+	CMY	-
E547	T	2010/06/06	Drainage	-	-	CMY	-
D218	O	2010/1/29	Bile	-	-	CMY	-
D219	O	2010/2/1	Ascites	-	-	CMY	-
E208		2009/09/19	Bile	+	+	CMY	-
E217		2009/10/08	Urine	-	-	CMY	-
E225		2009/10/23	Urine	-	-	CMY	-
E232	L	2009/12/13	Discharge	-	-	-	-
E233	L	2009/12/14	Discharge	-	-	-	-
B207		2009/10/08	Sputum	-	+	DHA	-
E210	P	2009/09/21	Bile	-	+	CMY	-
E537		2010/05/15	Discharge	-	-	CMY	-
E554		2010/06/18	Drainage	-	-	CMY	-

Figure 4. Dendrogram of *Xba*I digested chromosomal DNA of 48 carbapenem resistant *K. pneumoniae*, with culture date (CDATE), β-lactamase (*bla*) ESBL (SHV and CTX-M), AmpC, and carbapenemase. Letters under Patient indicate isolates from the same patient.



MIRLNO	Patient	CDTATE	Specimen	SHV	CTX-M	AmpC	Carbapenemase
B209		2009/10/04	Urine	+	-	-	-
E236		2009/12/21	Urine	-	-	-	-
B295		2010/03/30	Sputum	+	-	-	-
E207		2009/09/16	Blood	-	+	DHA	-
E227		2009/11/03	Pus/Wound	-	-	CMY	-
E213		2009/09/30	Urine	-	+	DHA	-
E223		2009/10/19	Urine	-	+	DHA	-
E231	K	2009/12/10	Liver	-	+	DHA	-
E502		2010/03/31	Blood	-	+	DHA	-
E286	N	2010/03/16	Bile	-	+	DHA	-
E555		2010/06/19	Bile	-	+	DHA	-
E288		2010/03/27	Bile	-	+	DHA	-
E219	I	2009/10/14	Sputum	-	+	DHA	-
E220	I	2009/10/15	Sputum	-	+	DHA	-
E250	N	2010/02/02	Bile	-	+	DHA	-
E511	N	2010/04/15	Drainage	-	+	DHA	-
E527		2010/05/06	Drainage	-	+	-	-
B229	R	2009/11/26	Sputum	-	+	DHA	-
B231	R	2009/11/25	Sputum	-	+	DHA	-
E525		2010/05/03	Urine	-	-	DHA	-
E235		2009/12/15	Sputum	-	+	DHA	-
E248		2010/01/20	Pus/Wound	-	+	DHA	-
E243		2010/01/17	Sputum	-	+	DHA	-
E204	D	2009/09/06	Bile	-	+	-	IMP
E205	D	2009/09/06	Blood	-	+	-	IMP
E209	D	2009/09/19	Sputum	-	+	-	IMP
D221		2010/2/22	Urine	-	+	DHA	-
E284	V	2010/03/11	Urine	-	-	CMY	-
E540		2010/05/18	Sputum	-	-	CMY	-
E222	J	2009/10/19	Urine	-	-	CMY	-
E216	J	2009/10/06	Tip	-	-	CMY	-
E229		2009/12/03	Urine	-	-	CMY	-
E241	M	2009/12/31	Sputum	-	-	CMY	-
E239	M	2009/12/30	Urine	-	-	CMY	-
E240	M	2010/01/01	Urine	-	-	CMY	-
E501		2010/03/31	Blood	-	-	CMY	-
E510	V	2010/04/15	Sputum	-	-	CMY	-
E230		2009/12/03	Sputum	-	-	CMY and DHA	-
D217		2010/1/30	Bile	-	-	-	-
D228		2010/3/17	Urine	-	-	-	-
D227		2010/3/17	Urine	-	-	-	-
E244		2010/01/05	Urine	-	+	-	-
D208		2009/11/5	Urine	+	-	DHA	-
E553		2010/06/16	Sputum	-	-	DHA	-
D215		2010/1/8	Tip	-	-	DHA	-
E224	K	2009/10/23	Liver	+	-	-	-
E283	U	2010/03/08	Bile	-	+	-	-
E507	U	2010/04/09	Bile	-	+	-	-

Figure 5. Comparison of carbapenem resistance rates in *A. baumannii* from ICU of 6 hospitals (Excluding duplicate isolates)

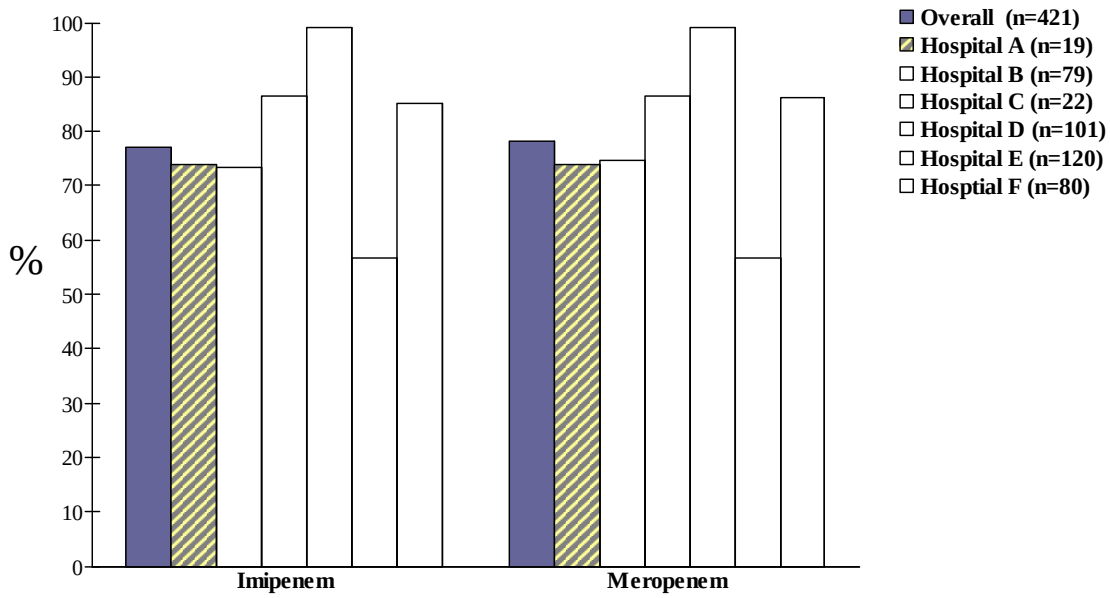
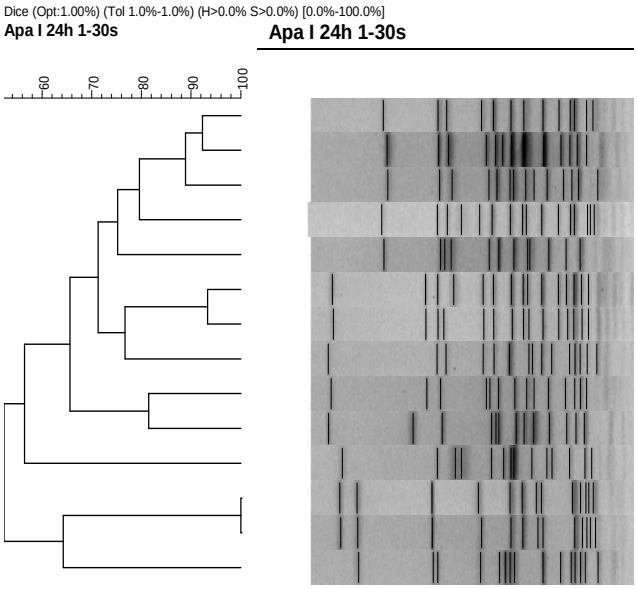


Figure 6A. Dendrogram of *Apa*I digested chromosomal DNA of 14 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (A). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.



Isolate #	CDATE	SOURCE TYPE	ST pattern	dpulicate
A009	103109	Wound/Pus	ACLTIM	
A016	031010	urine	ACLTIM	
A017	031510	resp.	ACLTIM	
A001	090409	Wound/Pus	_CLTIM	AA
A224	110109	urine	ACL_IM	
A002	090809	resp.	_CLTIM	AA
A007	100209	resp.	ACLTIM	
A013	121309	resp.	ACLTIM	
A214	092809	resp.	ACLTIM	
A236	120309	Wound/Pus	ACLTIM	
A259	022210	urine	_CLTIM	
A008	101309	blood	_CLTIM	
A015	011310	resp.	ACLTIM	
A014	011110	resp.	ACLTIM	

Figure 6B. Dendrogram of *Apa*I digested chromosomal DNA of 23 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (B). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.

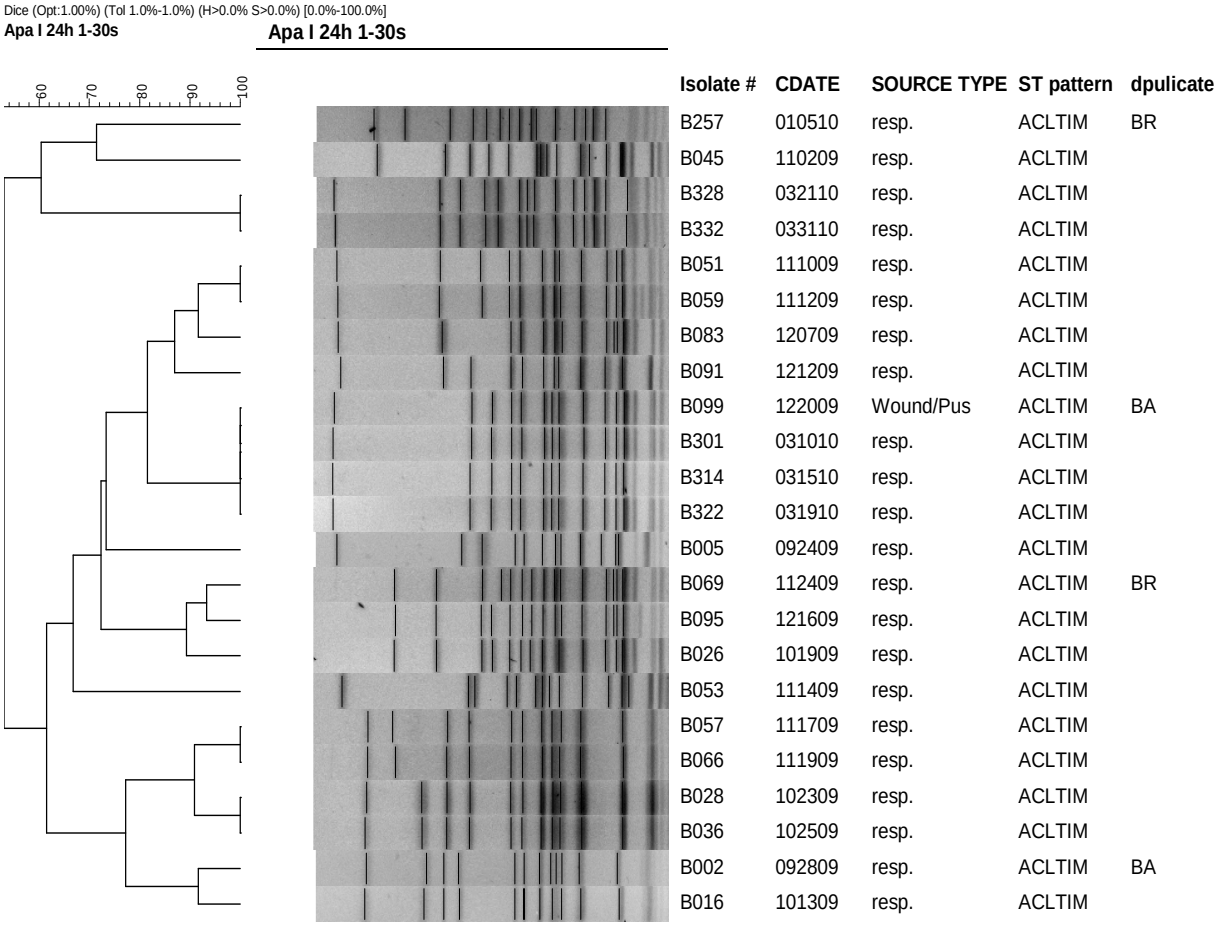
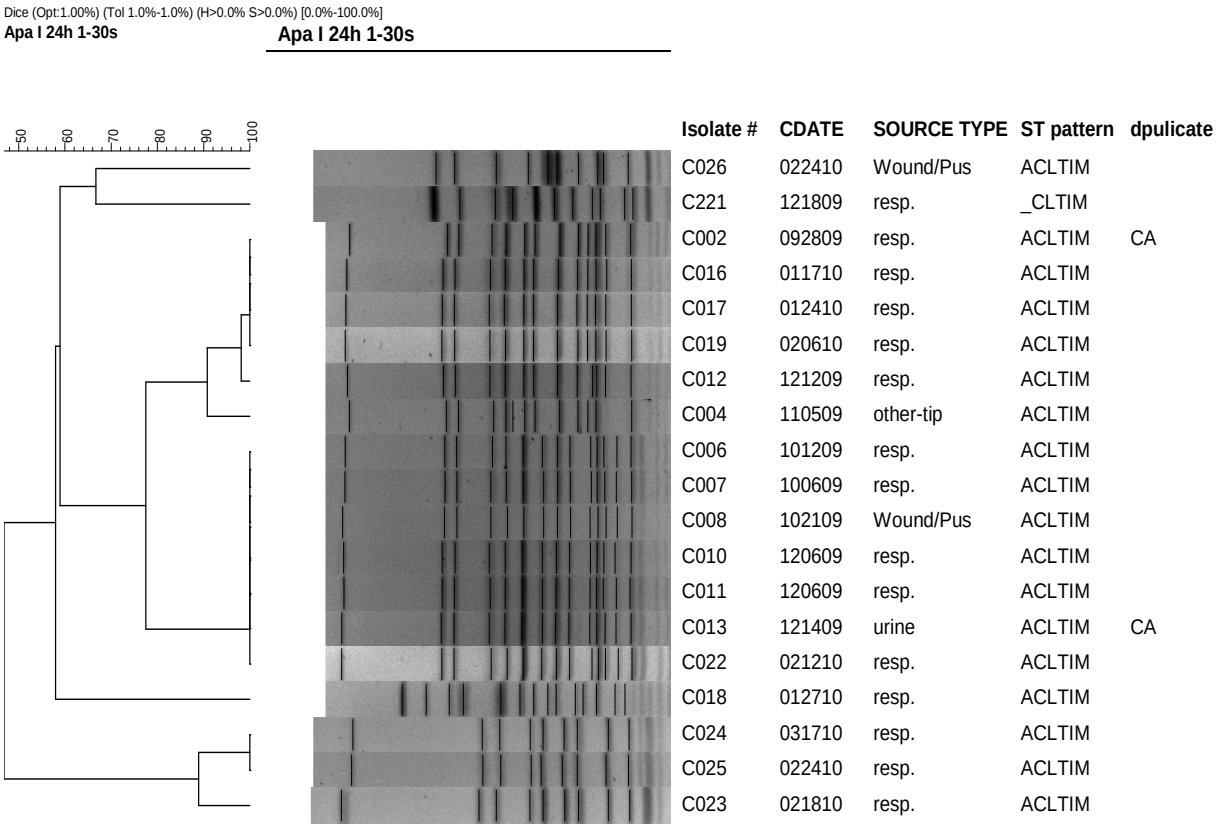
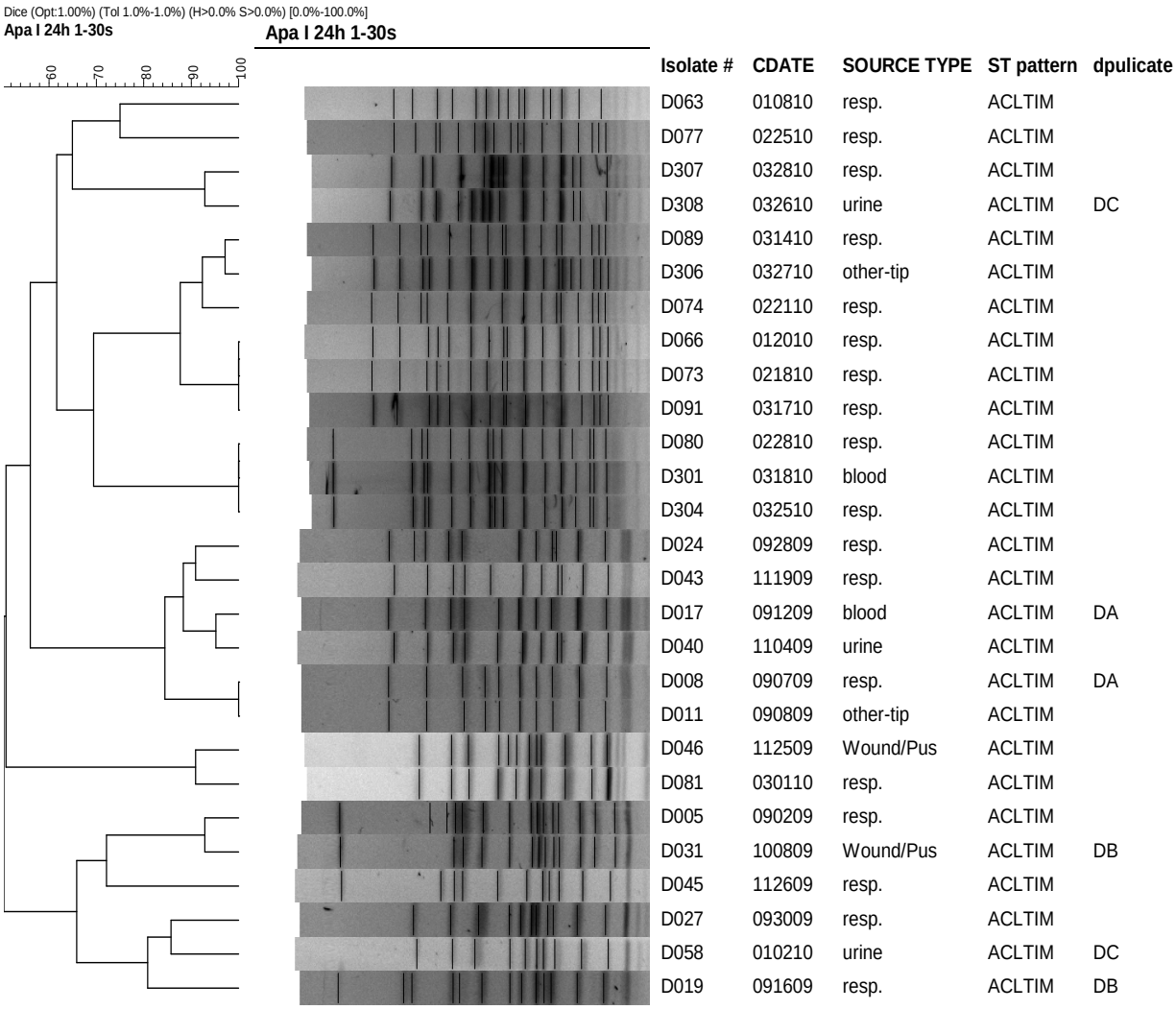


Figure 6C. Dendrogram of *Apa*I digested chromosomal DNA of 19 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (C). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.



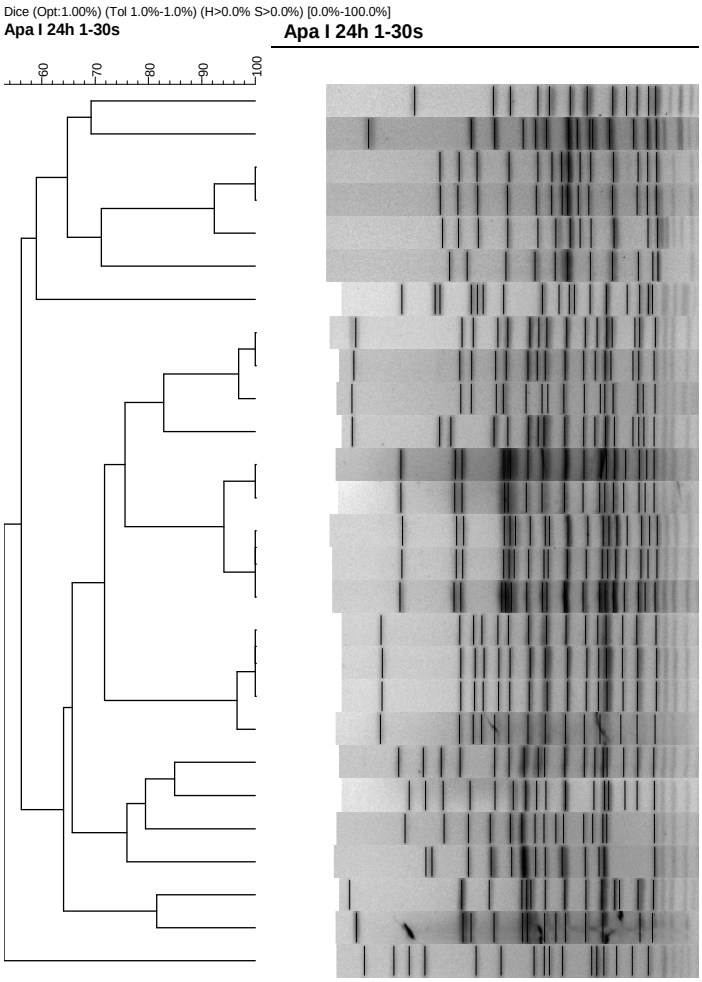
Isolate #	CDATE	SOURCE TYPE	ST pattern	dpulicate
C026	022410	Wound/Pus	ACLTIM	
C221	121809	resp.	_CLTIM	
C002	092809	resp.	ACLTIM	CA
C016	011710	resp.	ACLTIM	
C017	012410	resp.	ACLTIM	
C019	020610	resp.	ACLTIM	
C012	121209	resp.	ACLTIM	
C004	110509	other-tip	ACLTIM	
C006	101209	resp.	ACLTIM	
C007	100609	resp.	ACLTIM	
C008	102109	Wound/Pus	ACLTIM	
C010	120609	resp.	ACLTIM	
C011	120609	resp.	ACLTIM	
C013	121409	urine	ACLTIM	CA
C022	021210	resp.	ACLTIM	
C018	012710	resp.	ACLTIM	
C024	031710	resp.	ACLTIM	
C025	022410	resp.	ACLTIM	
C023	021810	resp.	ACLTIM	

Figure 6D. Dendrogram of *Apa*I digested chromosomal DNA of 27 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (D). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.



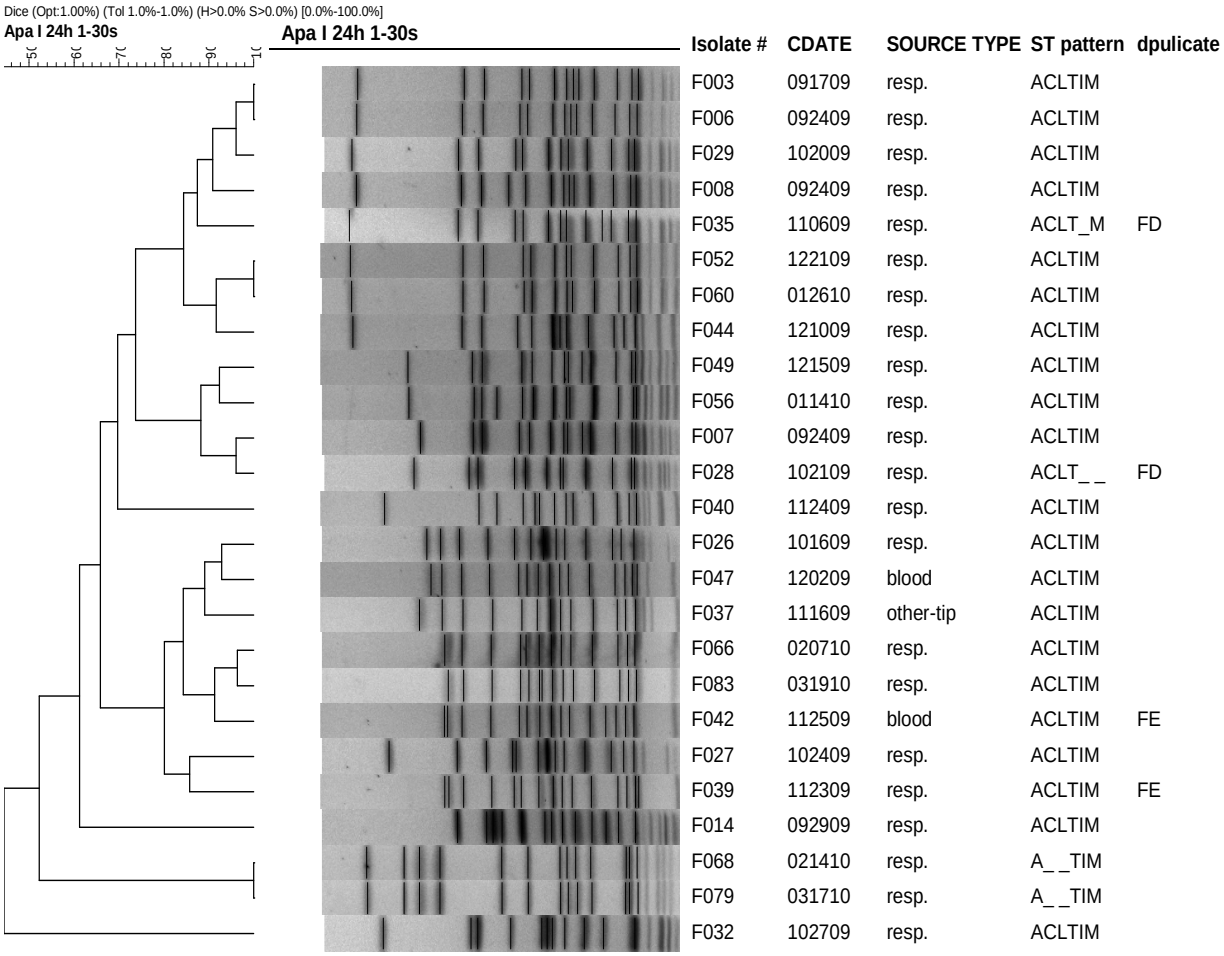
Isolate #	CDATE	SOURCE TYPE	ST pattern	dpulicate
D063	010810	resp.	ACLTIM	
D077	022510	resp.	ACLTIM	
D307	032810	resp.	ACLTIM	
D308	032610	urine	ACLTIM	DC
D089	031410	resp.	ACLTIM	
D306	032710	other-tip	ACLTIM	
D074	022110	resp.	ACLTIM	
D066	012010	resp.	ACLTIM	
D073	021810	resp.	ACLTIM	
D091	031710	resp.	ACLTIM	
D080	022810	resp.	ACLTIM	
D301	031810	blood	ACLTIM	
D304	032510	resp.	ACLTIM	
D024	092809	resp.	ACLTIM	
D043	111909	resp.	ACLTIM	
D017	091209	blood	ACLTIM	DA
D040	110409	urine	ACLTIM	
D008	090709	resp.	ACLTIM	DA
D011	090809	other-tip	ACLTIM	
D046	112509	Wound/Pus	ACLTIM	
D081	030110	resp.	ACLTIM	
D005	090209	resp.	ACLTIM	
D031	100809	Wound/Pus	ACLTIM	DB
D045	112609	resp.	ACLTIM	
D027	093009	resp.	ACLTIM	
D058	010210	urine	ACLTIM	DC
D019	091609	resp.	ACLTIM	DB

Figure 6E. Dendrogram of *Apa*I digested chromosomal DNA of 27 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (E). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.



Isolate #	CDATE	SOURCE TYPE	ST pattern	dpulicate
E328	031910	urine	ACLTIM	
E368	033010	resp.	ACLTIM	
E346	032510	resp.	ACLTIM	
E350	032510	resp.	ACLTIM	
E336	032210	resp.	ACLTIM	
E337	031910	other-tip	ACLTIM	
E321	031710	resp.	ACLTIM	
E020	091209	resp.	ACLT_ _	EF
E099	110209	other-sbs	ACLT_ _	EF
E081	101709	other-tip	ACLTIM	
E305	031510	resp.	ACLTIM	
E001	090309	resp.	ACLTIM	
E015	091309	resp.	ACLTIM	
E024	091409	resp.	ACLTIM	EA
E029	091509	other-sbs	ACLTIM	EA
E038	091709	Wound/Pus	ACLTIM	EA
E309	031310	urine	ACLTIM	ET
E317	031610	resp.	ACLTIM	
E318	031410	blood	ACLTIM	ET
E008	090709	resp.	ACLTIM	
E301	031210	resp.	ACLTIM	
E326	031810	resp.	ACLTIM	
E095	102909	Resp.	ACLTIM	
E049	092109	resp.	ACLTIM	
E304	031310	resp.	ACLTIM	
E004	090709	resp.	ACLTIM	
E059	092809	others	A_ _TIM	

Figure 6F. Dendrogram of *Apa*I digested chromosomal DNA of 25 ICU carbapenem-resistant *Acinetobacter baumannii* from hospital (F). CDATE, culture date, ST pattern, antimicrobial resistance pattern A, amikacin; C, ceftazidime, L, levofloxacin, T, piperacillin/tazobactam; I, imipenem; M, meropenem. Letters under duplicate indicate isolates were from the same patient.



Isolate #	CDATE	SOURCE TYPE	ST pattern	dpulicate
F003	091709	resp.	ACLTIM	
F006	092409	resp.	ACLTIM	
F029	102009	resp.	ACLTIM	
F008	092409	resp.	ACLTIM	
F035	110609	resp.	ACLT_M	FD
F052	122109	resp.	ACLTIM	
F060	012610	resp.	ACLTIM	
F044	121009	resp.	ACLTIM	
F049	121509	resp.	ACLTIM	
F056	011410	resp.	ACLTIM	
F007	092409	resp.	ACLTIM	
F028	102109	resp.	ACLT_ _	FD
F040	112409	resp.	ACLTIM	
F026	101609	resp.	ACLTIM	
F047	120209	blood	ACLTIM	
F037	111609	other-tip	ACLTIM	
F066	020710	resp.	ACLTIM	
F083	031910	resp.	ACLTIM	
F042	112509	blood	ACLTIM	FE
F027	102409	resp.	ACLTIM	
F039	112309	resp.	ACLTIM	FE
F014	092909	resp.	ACLTIM	
F068	021410	resp.	A_ _TIM	
F079	031710	resp.	A_ _TIM	
F032	102709	resp.	ACLTIM	

ATTACHMENT

MRSA Case Report Form