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台灣成人社區相關抗青黴素金黃色葡萄球菌移生之盛行率及危險因子，與其對院內血流感染預後之影響

The Prevalence of and Risk Factors for the Carriage of Community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and the Impact of CA-MRSA on the Prognosis of Nosocomial Bloodstream Infection Caused by MRSA Among Taiwanese Adults

王振泰

Jann-Tay Wang

指導教授：賴美淑　教授

季瑋珠　教授

Advisor: Mei-Shu Lai, Ph.D.

Wei-Chu Chie, Ph.D.

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本論文係王振泰君（學號 D94846002）在國立臺灣大學公共衛生學院預防醫學研究所完成之博士學位論文，於民國九十九年一月十二日承下列考試委員審查通過及口試及格，特此證明

口試委員：

李偉琳　賴美淑　（簽名）

（指導教授）

張上淳

楊采菱

方啓泰

系主任、所長　（簽名）

（是否須簽章依各院系所規定）

誌謝

能夠完成這份論文，心中著實百感交集。原本並未將博士學位放在人生規劃中，多虧了張上淳老師的鼓勵、太太的支持，才下定決心、鼓足勇氣，在離開學校 12 年之後，重拾學生生涯。回想過去以來，幾位老師的多方指導，深覺感念。

張上淳老師引領我進入抗青黴素金黃色葡萄球菌的研究領域，不但確立了我在研究上所需的臨床基礎，訓練我成為具有初步研究能力的研究者，更提供了許多研究機會給我。楊采菱老師是我實驗室工作的啟蒙者，讓我對原本完全陌生的分子生物學實驗，慢慢得以駕輕就熟。猶記得第一次上有關預防醫學的課程時，自己是和預防醫學多麼的扞格不入；在賴美淑老師和煦的目光和體諒的笑容裡，我終於能明白醫學之寬廣不限於臨床，終於能站在更高的角度看待事物，也終於能一窺預防醫學的堂奧。季瑋珠老師則總是指出我研究的盲點，點出研究的另一個可能角度，讓我能重新思考研究設計的適當性和合理性。方啟泰老師於我而言，亦師亦兄；他不僅帶著我看病人，帶著我做研究，帶著我寫文章，更是我生活上眾多雜事的請益對象，也是我情緒低落、灰心沮喪時的支持者。沒有老師們的教導，定然沒有今日我的成果。

除了良師，還要感謝一群熱心協助我的人們。在黃政華醫師、李文生醫師、廖俊星醫師的幫忙之下，我的研究才得以順利通過各醫院倫理委員會的審查，如願在各醫院中進行並完成。而實驗室裡，最要感謝的是美伶、家敏和雨薇，陪著我篳路藍縷的建立各種分子生物學相關實驗，從無到有、一無怨言；舒美、逸慈、含眉、雯倩幫忙收集菌株、鑑定分型，亦多所助益；麗茵則在統計與資料處理上，給了我莫大的幫助。另外，疾病管制局與國家科學委員會則是幕後的幫手，它們對我相關研究計畫的補助，讓我得以持續各項研究，順利獲得研究成果。

在讀博士班的過程中，我第三個小孩出生了，也經歷了人生第一次喪失至親

的苦痛。三個小孩的哭鬧與歡笑，是夜闌人靜、精疲力盡中，驀然給我心頭一絲甜美、激勵我無怨走下去的力量。父母雙親與外婆在電話一端的殷殷關懷與期盼，更給了我十足的勇氣。牽手惠玲甘為糟糠之妻，替我打理家中麻麻密密的一切瑣事，讓我無後顧之憂得以完成學業，又豈是一聲言謝就能道盡心中的感激？我自小隨著外公、外婆長大，外公卻在前年十月過世，沒來得及讓他看到我博士班畢業；在此謹以此篇論文，獻給教我劈柴生火、畜養栽植等生活技能，與堅忍無為、怡然自得等人生哲學的外公。

摘要

背景與目的：社區相關性抗青黴素金黃色葡萄球菌（community-associated methicillin-resistant *Staphylococcus aureus*，簡稱 CA-MRSA 菌株），自 1990 年代興起後，仍有許多流行病學及臨床上的問題尚未釐清；本論文乃針對下列三點加以研究：其一，社區成年人之 CA-MRSA 帶菌狀況與危險因子；其二，加護病房住院成年患者之 CA-MRSA 帶菌狀況與危險因子；其三，由 CA-MRSA 所引起的 MRSA 院內血流感染，是否有不同的死亡率。

研究方法：在我們的研究中，微生物學部分的採用如下一致的研究方法：針對培養出的金黃色葡萄球菌先進行青黴素的感受性測試，以確定其為 MRSA，而後測定其對各種抗生素的感受性，再以 multi-locus sequence typing、staphylococcal cassette chromosome *mec* (SCC*mec*) element 分型，辨別其是否為 CA-MRSA。在社區成年人之 CA-MRSA 帶菌狀況與危險因子的研究中，選擇在 2007 年 10 月 1 日到 2007 年 12 月 31 日的三個月間，參與職場健康檢查的成年人，篩檢其鼻腔的 MRSA 帶菌狀況；並收集其相關的人口學資料，生活習性，醫療機構、抗生素的暴露狀況等因素。在加護病房住院成年患者之 CA-MRSA 帶菌狀況與危險因子的研究中，利用在 2008 年 9 月 1 日至 2009 年 9 月 30 日，住在亞東紀念醫院內科加護病房與心臟血管加護病房的患者，對所有住進加護病房的患者，在剛住進的當天與之後的每三天採檢鼻腔拭子、腋下、喉嚨或痰液、與鼠蹊部進行細菌培養；並收集其相關的人口學資料，潛在性系統性疾病、相關醫療行為暴露、抗生素的暴露狀況等因素。在 MRSA 院內血流感染的研究中，利用在 2006 年 1 月 1 日至 2008 年 12 月 31 日，住在台大醫院、發生 MRSA 院內血流感染的患者，進行 MRSA 血流感染癒後的回溯性研究。

研究結果：在社區成年人之 CA-MRSA 帶菌狀況與危險因子的研究中，計有 3098

人接受篩檢，有 687 人帶有金黃色葡萄球菌，而其中有 111 人所帶的 MRSA 菌株屬於 CA-MRSA 菌株；社區中成年人 CA-MRSA 菌株帶菌的盛行率為 3.6%。相對於沒有金黃色葡萄球菌移生的人，家中有 7 歲以下的兒童、與一年內曾使用過抗生素的人，是 CA-MRSA 菌株移生的危險因子；相對於有 MSSA 移生的人，家中有 7 歲以下的小孩、過去一年內曾使用過抗生素的人，是 CA-MRSA 菌株移生的危險因子。而我們發現抽煙可以抑制 *S. aureus*（包含 CA-MRSA）在鼻腔的移生。

在加護病房住院成年患者之 CA-MRSA 帶菌狀況與危險因子的研究中，在計有 1906 位加護病房患者被篩檢，有 203 位被發現在一到加護病房時即帶有 MRSA，81 位患者被發現是在停留於加護病房中時，才新得到 MRSA。在 81 位新得到 MRSA 的患者中，有 31 人其分離出的 MRSA 菌株屬於 CA-MRSA 菌株。加護病房中心得到 CA-MRSA 菌株帶菌的發生率為每 1000 人日數 3.0 次。相對於沒有金黃色葡萄球菌移生的人，使用過 anti-pseudomonal penicillin 和 anti-fungals、以及使用過鼻胃管的患者，是新得到 CA-MRSA 菌株移生的危險因子；相對於新得到 HA-MRSA 移生的人，使用過 carbapenems 卻是不會新得到 CA-MRSA 菌株移生的保護因子。

在 MRSA 院內血流感染的研究中，總計有 308 次 MRSA 院內感染菌血症被納入分析、取得了 253 次菌血症的致病菌株。在 253 株致病菌株中，有 47 株屬於 CA-MRSA 菌株。菌血症發生後 14 天內的不分原因死亡率為 19.8%，30 天內為 30.5%。統計的結果發現，發生菌血症時有敗血性休克、血小板低下、以及菌血症發生 48 小時內沒有使用有效抗生素，是 14 天內死亡的危險因子。而發生菌血症時有敗血性休克、患有惡性腫瘤疾病、貧血、血小板低下、以及分離出的 MRSA 菌株之 vancomycin 最低抑菌濃度為 2 mg/L，是 30 天內死亡的危險

因子。而 CA-MRSA 菌株感染，則非影響預後的獨立因子

結論：總之，我們的研究釐清了 CA-MRSA 菌株在社區中、在加護病房中成年人移生的盛行率，指出了先前抗生素的使用是影響後續移生的重要危險因子、抽菸可以抑制 *S. aureus* 在鼻腔的移生；而 CA-MRSA 菌株侵入醫院環境後，造成院內菌血症感染，並不會導致更高的死亡率。我們應定期進行追蹤研究，以瞭解社區中 CA-MRSA 盛行率、分子分型、藥物感受性、以及其所引起之感染的預後變化。然而在目前，鑑定引起院內血流感染的 MRSA 是否為 CA-MRSA 似乎仍無其必要性。

關鍵字：社區相關性抗青黴素金黃色葡萄球菌，移生，菌血症，危險因子

Abstract

Background: Since the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), some epidemiologic and clinical issues caused by it remain unresolved. Our studies were focus on the following three issues: the prevalence of and risk factors for carriage of CA-MRSA among community healthy adults, the prevalence of and risk factors for carriage of CA-MRSA among intensive care unit (ICU) adult patients, and the impact of CA-MRSA on mortality of nosocomial MRSA bloodstream infection.

Materials and methods: The microbiologic studies of all *S. aureus* isolates were as following: identification of methicillin resistance by drug susceptibility, then multi-locus sequence typing and typing for staphylococcal cassette chromosome *mec* (SCC*mec*) element for all MRSA isolates to determine whether they belonging to CA-MRSA or not. In the study about the prevalence of and risk factors for carriage of CA-MRSA among community healthy adults, we enrolled adults attending mandatory health examination at 3 medical centers and sign the informed consents during Oct. 1, 2007 to Dec. 31, 2007 to screen their nasal carriage of MRSA. In the study about the prevalence of and risk factors for carriage of CA-MRSA among ICU adult patients, we enrolled all patients staying in two ICUs at the Far Eastern Memorial Hospital from Sep. 1, 2008 to Sep. 30, 2009 to clarify the prevalence and risk factors of carriage of CA-MRSA among ICU adult patients. Surveillance cultures from nostril, sputum or throat, axillae, and inguinal area were taken on all patients when

they just arrived at ICUs and then every three days. In the study about the mortality of nosocomial MRSA bloodstream infection, we retrospectively analyzed all adult patients hospitalized at National Taiwan University Hospital with nosocomial MRSA bloodstream infection from Jan. 1, 2006 to Dec. 31, 2008 to clarify the impact of CA-MRSA on prognosis of nsocomial MRSA infection.

Results: In the study about the prevalence of and risk factors for carriage of CA-MRSA among community healthy adults, 3098 people were enrolled, and 687 were found to carry *S. aureus*, among whom 111 carried CA-MRSA isolates. The prevalence of CA-MRSA carriage was 3.6%. Presence of household member aged ≤ 7 years, and use of antibiotics during the past year were the risk factors for carriage with CA-MRSA in comparison with both those without carriage of *S. aureus* and those with carriage of MSSA. Smoking was a significant factor inhibiting the nasal carriage of *S. aureus*.

In the study about the prevalence of and risk factors for carriage of CA-MRSA among ICU adult patients, 1906 patients were screened, and 203 patients were found to carry with MRSA before admission to ICU, while 81 were found to newly acquire MRSA during their stay in ICUs. Among the 81 patients, 31 carried with CA-MRSA isolates. The incidence rate of newly acquiring CA-MRSA carriage in ICU was 3.0 per 1000 patient-days. Prior usage of anti-pseudomonal penicillins and anti-fungals, as well as presence of nasogastric tube were independent risk factors for acquiring CA-MRSA during ICU stay compared to those without carriage of *S. aureus*. Prior usage of

carbapenems was a protective factor against acquiring CA-MRSA compared to those acquiring HA-MRSA during ICU stay.

In the study about mortality of nosocomial MRSA bloodsteam infection, 308 patients with nosocomial MRSA bloodstream infection were enrolled and 253 MRSA isolates, among which 47 belonged to CA-MRSA, were available for microbiologic studies. The Day 14 and Day 30 all-cause mortality were 19.8% and 30.5%, respectively. Septic shock, thrombocytopenia, and no effective antibiotics within 48 hours were independent risk factors for Day 14 mortality. Septic shock, having underlying malignancies, anemia, thrombocytopenia, and a causative MRSA isolate with a vancomyin minimum inhibitory concentration of 2 mg/L were independent risk factors for Day 30 mortality. In contrast, bloodstream infection caused by CA-MRSA isolates did not associated with a poorer outcome.

Conclusion: Prior usage of antibiotics is strongly associated with subsequent carriage of CA-MRSA among adults. Smoking could inhibit the nasal carriage of *S. aureus*. Nosocomial MRSA bacterermia caused by CA-MRSA was not associated with a poorer outcome. It is necessary to perform periodical surveillance on the prevalence, molecular types, drug susceptibilities, and impact on infection prognosis of CA-MRSA. However, it might not be indicated to identify whether the causative MRSA of nosocomial bloodstream infection was CA-MRSA or not at current time.

Keywords: community-associated methicillin-resistant *Staphylococcus aureus*, colonization, bloodstream infection, risk factor, CA-MRSA

Contents

Chapter 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the major pathogens causing nosocomial infections in Taiwanese hospitals since early 1990s. Its infections are usually associated with significant mortality and morbidity.[1] Considering the limitation of therapeutic choices to treat MRSA infection, therefore, controlling MRSA infection has become an important issue in clinical practices.[2]

Before 1990s, MRSA infection usually develops in patients with recent exposure to healthcare associated environment with known risks, such as hospitalization, recent operation, dialysis, residence in long-term care facility, and presence of invasive devices.[3] However, increasing reports about infections caused by MRSA among healthy personnel from community are noted during the past decade.[3] Therefore, MRSA infections are no longer exclusively found in patients with traditional risk factors, healthy personnel are also susceptible to MRSA infection at current time. MRSA infections are now classified as community-associated MRSA (CA-MRSA) infection and healthcare-associated MRSA (HA-MRSA) infection.[4] The former means the newly emerging disease entity that MRSA causes infection among previously healthy personnel. The later means the traditional one that MRSA caused

infection among patients with specific risk factors.

Subsequent microbiologic study demonstrates several differences between MRSA isolates causing tranditional HA-MRSA infection (HA-MRSA isolates) and emerging CA-MRSA infection (CA-MRSA isolates).[3, 4] The CA-MRSA isolates usually carry type IV or V staphylococcal cassette chromosome *mec* (SCC*mec*) element, an gene element determining resistance to methicillin and other antibiotics, and usually carry Panton-Valentin leukocidin (PVL) gene. However, HA-MRSA isolates usually carry type I, II, or III SCC*mec* element, and don't carry PVL gene.[4]

After the emergence of CA-MRSA isolates, we soon find an increasing of community-acquired infection caused by *S. aureus*, and the increase is majorly contributed by CA-MRSA.[3, 4] Later, CA-MRSA isolates are found to invade into healthcare-associated environment and cause an increasing proportion of CA-MRSA isolates among all MRSA isolates resulting in nosocomial infections.[3] Of special interest, it has been documented that CA-MRSA isolates replace the HA-MRSA isolates and become predominant in some hospitals.[3, 4]

All the above observation has implied that the CA-MRSA isolates have caused a great clinical impact, both in community and healthcare-associated settings.[3, 4] It is important to understand the clinical outcome of infection

caused by CA-MRSA isolates under such situation. In the community settings, we should compare the outcome of infection between those caused by CA-MRSA and methiciilin-susceptible *S. aureus* (MSSA). In the healthcare settings, we should compare the outcome of infection between those caused by CA-MRSA and HA-MRSA. Besides, prior study pointed out that patient with MRSA infection usually developed MRSA colonization before the infection.[3] Therefore, it is also important to detect the prevalence of and risk factors for CA-MRSA colonization among both inpatients and people in community setting.

Chapter 2. Literature review

1. Changing the concept of MRSA infection in clinical aspect

1.1 Traditional view of MRSA infection

Before the emergence of so-called CA-MRSA, nearly all MRSA infections were nosocomial origin, and developed especially in patients with specific risk factors such as receipt of systemic antimicrobial agents, residence in a long-term care facility, prior hospitalization into an acute care facility, use of central venous catheters or long-term vascular access devices, use of urinary catheter, presence of prior surgical procedures, and need of dialysis (Table 2–1).[5] This kind of disease entity is classified as HA-MRSA infection now as mentioned above.[4]

Despite indentified since 1961, MRSA was not a frequent adversary until 1980s.[1, 6] The increasing incidence of MRSA infections was most likely caused by growing impact of invasive devices, selection pressure from antibiotics, and older age as well as comorbidities of patients.[1, 7, 8] At current time, MRSA is one of the leading pathogens of nosocomial infections either in the U.S.A. or Taiwan.[9, 10]

Table 2–1. Risk factor for MRSA infections

Type of MRSA infections	Risk factors for Infections	
HA-MRSA[a]	1. Hospitalization, surgery, dialysis, or residence in a long-term care facility within past one year 2. Presence of permanent indwelled catheters	3. Presence of percutaneous medical devices 4. Previously known positive MRSA culture
CA-MRSA[b]	1. Children under 2 years 2. Participants of contact sports 3. Intravenous drug user 4. Men having sex with men 5. Military personnel 6. People in correctional facilities, residential homes, or shelters 7. Veterianarians, pet owners, and pig farmers 8. Aged adults older than 65 years 9. African Americans 10. Recent influenza-like illness or sever pneumonia 11. Concurrent skin and soft-tissue infection 12. History of colonization or recent infection with CA-MRSA 13. Known close contact with a person positive for CA-MRSA	

Abbreviation: HA-MRSA, healthcare-associated methicillin-resistant *S. aureus*; CA-MRSA, community-associated methicillin-resistant *S. aureus*

[a]Based on reference 5. [b]Based on reference 32–34, 36, 39–41.

The clinical spectrum caused by MRSA consists of skin and soft tissue infection (39.2%), lower respiratory tract infection (23.2%), bloodstream infection (including endocarditis) (22%), urinary tract infection, prosthetic device-related infection, and toxin-mediated diseases (which contains toxic shock syndrome, food poisoning, and staphylococcal scalded-skin syndrome.[11, 12] Several recent reports revealed a continuous increase in MRSA infections in hospitals.[13 – 17] Of most importance, MRSA infections, especially those with bloodstream infection, resulted in notable morbidity and mortality,[18 – 21] compared with infections caused by MSSA.[19, 20] The all-cause mortality rate on Day 30 after MRSA bloodstream infection was around 36%.[18 – 21] The risk factors for mortality included old age, delay in effective therapy, immunosuppressive status, advanced underlying diseases, presence of septic shock at presentation, and a minimum inhibitory concentration (MIC) to vancomycin equal to or over 2 mg/L (Table 2–2). [18 – 21] Old age, immunosuppressive status, and advanced underlying diseases implied the poor host general condition and therefore resulted in adverse outcomes. Presence of septic shock stood for advance severity of MRSA infections and could predict adverse outcomes. [18 – 21] A vancomycin MIC ≥ 2 mg/L meant a poorer response to vancomycin treatment.[21]

MRSA infections are also transmissible, spreading from person to person and from one hospital to another.[22, 23] Therefore, MRSA has caused numerous epidemics in many hospitals.[11] In order to identify epidemic strains, several methods of molecular typing have been developed.[24] Of which, pulsed-field gel electrophoresis (PFGE), *spa* typing, and multilocus sequence typing (MLST) are most popular and important.[25 – 26]

Table 2–2. The risk factors for mortality among patients with MRSA infection

Strains of MRSA	Risk factors for mortality
HA-MRSA[a]	Old age, delay in effective therapy, advanced underlying diseases, immunosuppressive status, presence of shock, a vancomycin MIC ≥ 2 mg/L
CA-MRSA[b]	Old age, presence of shock at presentation, thrombocytopenia with a level < 100,000 cells/mL

[a]Based on references 18–21. [b]Based on reference 4

1.2 New concept of MRSA infection: emergence of CA-MRSA

As described above, before the late 1990s, nearly all MRSA infections occurred in healthcare settings (Table 2–1).[5] In 1993, MRSA isolates with unique genetic features were reported among infected western Australian aborigines without any history of contact with the health care system.[27] In

addition, there were four lethal episodes of MRSA infections identified in four American children, who haven't had any traditional risk factors for MRSA infections, in 1999.[28] In addition to the different clinical settings from traditional MRSA infections, the MRSA isolates isolated from these events also have different phenotypic and genetic features from previous nosocomial MRSA (HA-MRSA) isolates.[29, 30] In brief, the CA-MRSA isolates carry different type SCC*mec* element and are not multidrug resistant (usually susceptible to tetracycline, clindamycin, erythromycin, fluoroquinolones, and trimethoprim/sulfamethoxazole). HA-MRSA isolates carry type I, II, or III SCC*mec* elements; while the CA-MRSA isolates carry type IV or V.[4, 30, 31]

Thereafter, several other studies reported the similar findings and CA-MRSA strains were found to emerge in several countries over the world, including Canada, the U.S.A., Mexico, Brazil, Argentina, Western Europe (Greece, Finland, France, Germany, Netherlands, Norway, Sweden, Denmark, and Spain), Saudi Arabia, India, Japan, Korea, Hong Kong, Taiwan, Australia, and New Zealand.[32 – 35] Initially, CA-MRSA was usually found to cause infections among young children, especially those younger than two years, and the most clinical syndrome was skin and soft tissue infections.[36] Later, necrotizing pneumonia was also found to be a frequent disease caused by

CA-MRSA in young children.[32, 33] Because of the characteristics of tissue destruction and necrosis, the isolated CA-MRSA strains were further analyzed and found to usually carry the virulent gene of PVL,[32 – 34, 36, 37] whose product had the activity of leukocytolysis and had been epidemiologically associated with soft tissue infection.[38] Till now, although the definition of CA-MRSA is still blurring, the presence of SCC*mec* type IV or V and PVL have been useful molecular markers of CA-MRSA strains (Table 2–3).

Table 2–3. Suggested surrogates differentiating HA-MRSA from CA-MRSA[a]

Surrogate	HA-MRSA	CA-MRSA
Antibiogram	Resistant to various class of antibiotics other than β-lactams	Susceptible to antibiotics other than β-lactams
SCC*mec* typing	Type I, II, and III	Type IV or V
PVL gene	Absence	Presence

[a]Based on references 4, 30–34, 36, and 37.

Thereafter, CA-MRSA can cause community-associated infections among other patient populations, including athletes (mainly participants in contact sports), intravenous drug user, men who have sex with men, military personnel, persons living in correctional facilities, residential homes, or shelters,

veterinarians, pet owners, pig farmers, adult with age over 65 years, the African Americans, recent influenza-like illness and/or severe pneumonia, concurrent skin and soft-tissue infection, history of colonization or recent infection with a CA-MRSA strain, known close contact (in same household) with a person colonized and/or infected with MRSA (Table 2–1).[32–34, 36, 39–41] The risk factors associated with mortality due to CA-MRSA infection include old age, presence of shock at presentation, and thrombocytopenia with a level < 100,000 cells/mL at presentation (Table 2 – 2).[4]

In early 2000s, CA-MRSA isolates even invaded into healthcare-associated environment and caused infection among hospitalized patients who had traditional risk factors for HA-MRSA infection.[41–43] Therefore, CA-MRSA isolates are now able to cause nosocomial infection just as HA-MRSA isolates used to be.

2 Microbiology of *Staphylococcus aureus*: emphasis on the differentiation between CA-MRSA and HA-MRSA isolates

2.1 Basic description

Staphylococcus aureus belongs to the family of Microccaceae. Under Gram stain, it appears as gram-positive cocci in clusters.[44] Unlike other

staphylococcus species, *S. aureus* has the characteristics of coagulase, mannitol fermentation, positive deoxyribonuclease tests, and gold pigmentation of its colony.[45] Its hereditary materials include a circular chromosome, which contains about 2800 base-pairs, prophages, plasmids, and transposons.[11, 44] Its virulence and resistance to antibiotics are encoded by genes located on chromosomes and extra-chromosomal elements, such as the plasmids.[46] These genes are transferrable between staphylococcal strains, species, or even other gram-positive bacterial species.[47]

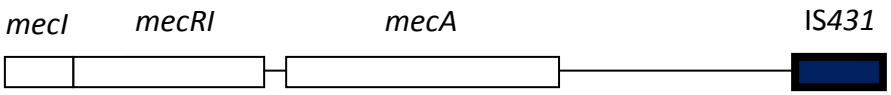
Some important chemicals of *S. aureus* play important roles in its infections and colonization in human beings. These include polysaccharidic capsule,[48] protein A,[11] peptidoglycan,[49] cytotoxins,[50, 51] , enterotoxin A – D,[52] exfoliatin A & B,[11] toxic shock toxin-1,[44, 53] and PVL.[38] PVL has the activity of leukocytolysis and has been epidemiologically associated with soft tissue infection and necrotizing pneumonia.[38]

S. aureus has developed resistances to all available antibiotics, including penicillin,[54–56] methicillin,[57] glycopeptides,[58–63] antibiotics of macrolide-licosamide-streptogramin B group,[64, 65] quinolones,[66, 67] tetracyclines,[42, 43] aminoglycoside,[44 – 46] sulfa drugs,[44] rifampin,[68, 69] chloramphenicol,[44] fusidic acid,[70, 71] linezolid,[72–74] and daptomycin.[54] Among

these resistances, methicillin resistance is of special clinical interest because once present, these *S. aureus* isolates are considered to be resistant to all of the β-lactams, which are the most frequently used antibiotics in clinical practices at current time.[44]

The methicillin resistance of *S. aureus* is mediated by the production of an additional and modified penicillin-binding protein (PBP), PBP-2a (or named as PBP-2'), which leads to much decreased affinity of methicillin and all other β-lactams.[75, 76] PBP-2a is encoded by the *mecA* gene.[77] Later research demonstrated a *mecA* gene complex consists of the *mecA* gene and other regulatory genes, *mecI* and *mecRI* (figure 2–1).[78] During the same time period, other researches revealed that there is an additional DNA (also named as mec DNA) about 30 kb in size, which carries *mecA* gene complex and there is no allelic equivalence found in methicillin-susceptible *S. aureus* (MSSA), in MRSA chromosome.[79, 80] This additional DNA is first cloned and analyzed in 1999, and is then named as SCC*mec* element.[78] The most important components in the SCC*mec* element include the *ccr* and *mecA* gene complex.[81] By the typing and grouping of *ccr* and *mecA* gene complex, there are five major types of SCC*mec* elements found till now.[82] The typing of SCC*mec* element is also an important clue to differentiate CA-MRSA isolates from HA-MRSA isolates.[30, 31]

Figure 2 – 1. The *mec*A gene complex



2.2 Laboratory methods to differentiate CA-MRSA from HA-MRSA isolates

As mentioned above, some microbiologic features, including typing of SCC*mec* element and presence or not of PVL gene, differentiate CA-MRSA from HA-MRSA.[4, 30–34, 36, 37] In addition, CA-MRSA isolates also belongs to different sequence types determined using MLST from HA-MRSA.[4]

Typing of the SCC*mec* element can be identified using polymerase chain reaction (PCR) methods to detect different types of two major components in the SCC*mec* element, *ccr* and *mecA* gene complex.[81–83] There are three types of *mecA* gene complex (class A – C) and 5 types of *ccr* complex (type 1 – 5).[83] Type I SCC*mec* consists of class B *mecA* gene complex and type 1 *ccr* complex, type II SCC*mec* consists of class A *mecA* gene complex and type 2 *ccr* complex, type III SCC*mec* consists of class A *mecA* gene complex and type 3 *ccr* complex, type IV SCC*mec* consists of class B *mecA* gene complex and type 2 *ccr* complex, and type V SCC*mec* consists of class C *mecA* gene complex and type 5 *ccr* complex (Table 2–4).[83]

Table 2–4. Current SCC*mec* types (Reference 83)

SCC*mec* type	*mecA* gene complex	*ccr* gene complex
I	Class B	Type 1
II	Class A	Type 2
III	Class A	Type 3
IV	Class B	Type 2
V	Class C	Type 5

The presence of PVL gene also can be determined by PCR method, which has been provided by Lina et al.[84] The MLST is based on the neucleotide sequence results of 7 housekeeping gene, including *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL* in *S. aureus*.[26] The work starts with amplification by seven-pair primers of these 7 housekeeping genes. The 7 amplicons are then sequenced to determine each neucletode sequence. The neucleotide sequences are in turn compared with those in the databank. Therefoe, all unique sequences are assigned corresponding allele numbers and combined into an allelic profile and then assigned a sequence type (ST).[26] MLST is an ideal long term and global epidemiologic tool because the accumulation of nucleotide changes in housekeeping genes is a relatively slow process and the allelic profile of a bacterial isolate is sufficiently stable over time. In addition, by means of the convenience of internet and other softwares, the result of MLST can be easily

compared inter-laboratories.[26]

3 Epidemiology study of CA-MRSA in the U.S.A. and other countries

The emergence of CA-MRSA results in two clinical challenges immediately: First is the increase of community-acquired *S. aureus* infections, and that CA-MRSA contributes much more than MSSA to this increase (Table 2 – 5).[85–87] King et al reported that among 389 episodes of community-acquired skin and soft-tissue infection caused by *S. aureus*, 72% of them were due to MRSA.[85] Moran et al reported that among 422 patients with community-acquired skin and soft-tissue infections, 320 (76%) were caused by *S. aureus*, and 59% were caused by MRSA.[86] Kaplan et al reported that the number of community-acquired infections caused by *S. aureus* increased from 771 to 1562 during a three-year period, and MRSA contributed 81.2%.[87] The similar finding were observed in Korea, Sigapore, Greece, Austria, Finland, and the United Kindom.[88–93] However, the predominant strains of CA-MRSA circulating in those countries were different form those in the U.S.A..[94–96]

The second challenge is about the choice of antibiotics while facing a patient with community-acquired *S. aureus* infection. As described above, MRSA accounts for the majority of community-acquired *S. aureus* infection in

the clinical disease of skin and soft-tissue infection.[85–87] Antibiotics of β-lactams, which are the agents most frequently prescribed for community-acquired infections,[97] are not actively against MRSA. Should clinicians change their practice and prescribe antibiotics recommended for CA-MRSA? Although a clinical study revealed that inappropriately initial treatment did alter the outcomes of skin and soft-tissue infections caused by CA-MRSA,[86] the consumption of antibiotics recommended for CA-MRSA in treating skin and soft-tissue infections have increased in the U.S.A..[97]

Subsequently, another clinical impact by CA-MRSA occurred. It is the emergence of CA-MRSA infections among hospitalized patients with risk factors for HA-MRSA infection. This phenomenon has been documented by several studies (Table 2 – 6).[41–43] The Center for Disease Control and Prevention (CDC, U.S.A.) investigator found that 17.7% of hospital-associated MRSA infections were caused by CA-MRSA strain during July 2004 to December 2005 in the U.S.A..[41] Seybold reported that 57% of nosocomial MRSA bloodstream infection was caused by CA-MRSA isolates in 2004 at the Grady Memorial Hospital.[42] Maree et al also described that the proportion of CA-MRSA among all nosocomial MRSA infections increased from 17% in 1999 to 56% in 2003 ($p<0.0001$).[43] All these studies emphasized that CA-MRSA

strains has invaded into the healthcare settings, replaced the previous HA-MRSA strains, and became the major strains causing nosocomial MRSA infections. The similar findings were also noted in Korea, Asutria, and the United Kindom.[88, 91, 93] In these studies, it was also found that patients with nosocomial infection caused by CA-MRSA strains were younger, having a positive culture taken earlier in the hospital, more likely to have concurrent skin and soft-tissue infection, and more likely to be drug abusers than those caused by HA-MRSA strains.[42, 43] On the other hand, patients with nosocomial infection caused by HA-MRSA strains were more likely to have lived in a long-term care facility during the past one year, and more likely to have histories of antimicrobial use during the past one year.[42]

In addition, as colonization by CA-MRSA is a risk factor for subsequent CA-MRSA infections, several studies have been conducted to detect the prevalence of CA-MRSA infection in the community settings.[95 – 100] For children without any risk factors, the colonization rates range from 0.2% to 2.2%.[95 – 98] In addition, Creech et al reported that the nasal MRSA colonization rate among healthy children increased from 0.8% in 2001 to 9.2% in 2004.[99] In the population anticipated the National Health and Nutrition Examination Survey (NHANES), the MRSA colonization rate was 0.84%.[100] As the colonization rate

of CA-MRSA among people from communities increased, it could be expected that the incidence of community-acquired infections caused by CA-MRSA would continue to increase also in the near future.

Table 2 – 5. The proportion of MRSA among community-acquired infections caused by *S. aureus*: summary of recent studies.

Study period	Study site	No. of cases	Disease Patterns	Proportion of MRSA	Reference number
08/01/2003 – 11/15/2003	Atlanta, U.S.A.	389	SSTI	72%	85
08/01/2004 – 08/31/2004	Multicenters U.S.A.	422	SSTI	76%	86
08/01/2001 – 07/31/2002	Texas, U.S.A.	772	SSTI & others[a]	71.5%	87
08/01/2002 – 07/31/2003	Texas, U.S.A.	1245	SSTI & others	73.5%	87
08/01/2003 – 07/31/2004	Texas, U.S.A.	1562	SSTI & others	76.4%	87

Abbreviation: SSTI, skin and soft-tissue infection.

[a]Others include abscesses, bloodstream infection, empyema, endocarditis, lymphadenitis, meningitis, osteomyelitis, peritonitis, pneumonia, septic arthritis, and septicemia.

Table 2 – 6. The proportion of CA-MRSA among all MRSA causing nosocomial infections: summary of recent studies.

Study period	Study site	No. of cases	Disease Patterns	Proportion of CA-MRSA	Reference number
07/01/2004 – 12/31/2005	Multicenters, U.S.A.	7566	Invasive infections[a]	17.7%	41
7.5 months in 2004	Atlanta, U.S.A.	132	Bloodstream infection	57%	42
01/01/1999 – 12/31/1999	Los Angeles, U.S.A.	52	All	17%	43
01/01/2000 – 12/31/2000	Los Angeles, U.S.A.	57	All	19%	43
01/01/2001 – 12/31/2001	Los Angeles, U.S.A.	65	All	28%	43
01/01/2002 – 12/31/2002	Los Angeles, U.S.A.	47	All	43%	43
01/01/2003 – 12/31/2003	Los Angeles, U.S.A.	63	All	56%	43
01/01/2004 – 12/31/2005	Los Angeles, U.S.A.	68	All	52%	43

[a]Including bloodstream infection, pneumonia, cellulitis, osteomyelitis, endocarditis, and septic shock.

4 Epidemiology study of CA-MRSA in Taiwan

The earliest report related CA-MRSA infections in Taiwan was also from

the observation in pediatric patients.[104] Wang et al reported a case series of 19 pediatric patients (range of age, 7 months – 10.5 years) with MRSA infection while without any traditional risk factors for MRSA infection.[104] All the 19 patients presented with skin and soft-tissue infections. All 17 available MRSA isolates from these 19 patients were of ST59 and carried the PVL gene. They were unlike the HA-MRSA strains in Taiwan, which were usually of ST241 and ST239, had no PVL, and carried type III SCC*mec* element.[66, 105] At that time, only three of these 17 MRSA isolates carried type IV SCC*mec* element. The SCC*mec* elements of the other 14 isolates can't be identified (Later, these 14 isolates were found to carry a novel type V SCC*mec* element, named V_T.[106]). Almost during the same period, Chen et al also reported 32 pediatric patients with CA-MRSA infection (also all were skin and soft-tissue infection) while without any traditional risk factors for MRSA infection.[107] A subsequent microbiological study revealed these CA-MRSA strains belonged to ST59, and carried either type IV or a novel type, type V_T, SCC*mec*.[107] Nearly every isolate of CA-MRSA belonging to ST59-V_T carried the PVL gene; however, isolates of ST59-IV were not.[107, 108] In addition, about 35.5% of the pediatric patients with CA-MRSA infection had pneumonia and about 6.5% had central nervous system infection.[108] However, the mortality rate and risk factors for mortality

associated with CA-MRSA infection in the pediatric patients were not clearly addressed.

Therefore, the CA-MRSA strains in Taiwan are different from those in the U.S.A. by the molecular typing. In addition, isolates of CA-MRSA in Taiwan were more resistant to antibiotics of non-β-lactams than those from the U.S.A., especially in terms of clindamycin and erythromycin.[106] CA-MRSA isolates in Taiwan were nearly 100% resistant to macrolides and clindamycin, about 10% resistant to aminoglycosides, and various percentage resistant to tetracycline.[106, 109]

The CA-MRSA infection in Taiwanese adults was first noted in 2001.[109] The report was based on a prospective study focusing on patients presented with bloodstream infection at the Department of Emergency at National Taiwan University Hospital (NTUH) since 2001 (Chen SY, unpublished data). In the study by Wang et al, the community-acquired *S. aureus* bloodstream infection increased from 32 episodes in 2001 to 47 episodes in 2006.[109] Among these episodes, CA-MRSA contributed 3.1% in 2001 and 19.1% in 2006. All the CA-MRSA isolates belonged to ST59. The all-cause 30-day mortality rate was not different between patients with CA-MRSA bloodstream infection and those with CA-MSSA bloodstream infection (p=0.582).[109] This study and the study on

pediatric patients both revealed that the incidence rate of CA-MRSA infection is increasing in the community setting in Taiwan.[108, 109]

The other issue of CA-MRSA in Taiwan is whether these strains (ST59-IV and ST59-V_T) would spread into hospital environment or not. Huang et al reported that 80.6% of the MRSA isolates causing nosocomial bloodstream infection belonged to ST239 or ST241 and only 16.9% belonged to ST59 in 2001 and 2002 at the Chang Gung Memorial Hospital. However, only 42.7% belonged to ST239 or ST241 while 27.4% belonged to ST59 in 2004 and 2005.[110] In the study conducted by Wang et al, the predominant MRSA strain causing nosocomial bloodstream infection at the NTUH was ST254 (45%) in 1990, ST241 (55%) in 1995, ST239 (55%) in 2000, and ST59 (48.3%) in 2005.[17] Both studies demonstrated that CA-MRSA in Taiwan has invaded the hospital environment and replaced the prior HA-MRSA strains to become the predominant microbe.[17, 110]

The prevalence of CA-MRSA colonization in the community among children in Taiwan has been well studied.[106, 111 – 113] However, the prevalence rates of CA-MRSA colonization among healthy adults and among hospitalized patients remained unclear in Taiwan. Lu et al first screened 987 two- to 18-year-old schoolchildren in southern Taiwan in 2001 and detected a

prevalence of nasal carriage with CA-MRSA of 2.9%.[111] Huang et al screened 262 three- to 12-year-old school children in northern Taiwan during 2001 to 2002 and got a nasal carriage rate of CA-MRSA of 1.5%.[112] Boyle-Vavra et al also screened 640 school children in northern Taiwan when they visited health care in 2003 and got a nasal carriage rate of CA-MRSA of 5%.[106] Huang et al again screened 3046 two-month to 5-year-old children all over Taiwan when they visited health care from July 2005 to October 2006 and got a nasal carriage rate of CA-MRSA of 7.2%.[113]

In conclusion, the colonization pool of CA-MRSA among health children increased during the past decade in Taiwan. In addition, the clinical burden of CA-MRSA also increased among both adults and children population in Taiwan.

5. The unsolved issues about CA-MRSA in Taiwan

As described above, some issues related to CA-MRSA remain unsolved in Taiwan. First, the prevalence of nasal carriage of CA-MRSA among healthy adults and its risk factors are still unclear. Because history of carriage with CA-MRSA is a risk factor for subsequent CA-MRSA infection,[36] understanding the CA-MRSA colonization rate among healthy adults might be helpful in

estimating the disease burden of CA-MRSA infection among healthy adults. In addition, to control the spread of CA-MRSA among healthy adults in the community settings is possible only after the risk factors for acquisition of CA-MRSA are indentified.

Second, the prevalence of carriage of CA-MRSA among hospitalized patients, especially those ever stayed in intensive care units (ICUs), is also not clear. As more and more nosocomial MRSA infections are caused by CA-MRSA strains not HA-MRSA strains, to discover the colonization rate of and risk factors for acquiring CA-MRSA (compared to that of HA-MRSA) among hospitalized patients is also clinically relevant.

Third, CA-MRSA was considered more virulent than HA-MRSA because it could cause infection among previously healthy people.[5] Whether nosocomial infections caused by CA-MRSA strain lead to more mortality than those caused by HA-MRSA and the risk factors for this mortality are another important clinical issue.

In order to understand the clinical impact by CA-MRSA isolates in Taiwan more comprehensive, all the three issues should be answered.

6. Studies to investigate the unresolved issues in Taiwan

The following studies were designed and conducted to answer the three issues about CA-MRSA in Taiwan. The microbiologic methods to identify the CA-MRSA isolates were the same in the three studies.[83, 84] The first study is a cross-sectional one using nasal swab culture to identify nasal carriage of CA-MRSA isolates among health adults (age > 18 years), who attended health examination at three medical centers during a 3-month period. Demographic data and medical exposure were collected using a standardized questionnaire. The prevalence rate of and risk factors for nasal carriage of CA-MRSA were then determined.

The second study was a prospective one using active surveillance culture to identify carriage of CA-MRSA isolates among adult patients in ICUs. The active surveillance cultures were taken on all patients when they just arrived at ICUs and then every three days during a one-year period. The culture sites included nostril, throat or sputum (those who were intubated), axillary, inguinal area, and wound. A standardized case report form was used to collect demographic and medical data. The prevalence rate of, incident rate of newly acquiring, and risk factors for CA-MRSA colonization were then determined.

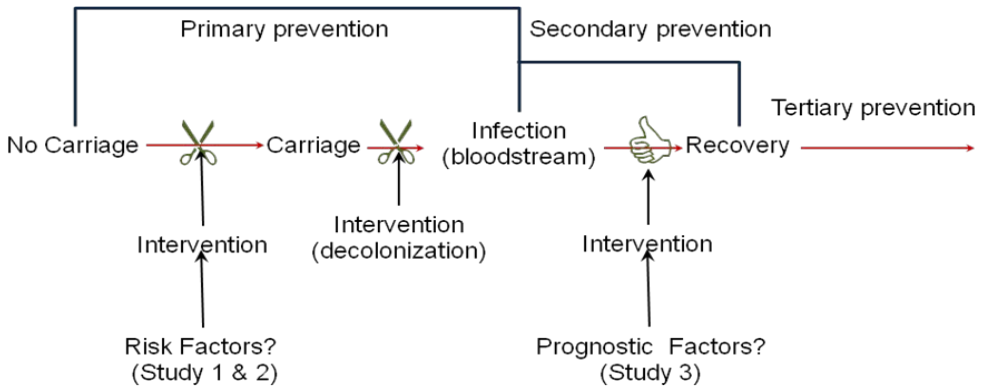
The third study was a retrospective one to analyze the clinical outcome of nosocomial MRSA bloodstream infection at NTUH during a 3-year period. The

causative MRSA isolates were obtained via the bacterial bank in the Department of Laboratory Medicine at NTUH. A standardized case report form was used to collect demographic and clinical data recorded in medical charts. All-cause Day 14 and Day 30 mortality were determined, and risk factors for mortality, including the impact of CA-MRSA on outcome, were also identified.

Chapter 3. Materials and methods

1 Study framework

Figure 3 – 1. Study framework



Study 1: the prevalence of and risk factors for carriage of CA-MRSA among community healthy adults
Study 2: the prevalence of and risk factors for carriage of CA-MRSA among ICU adult patients
Study 3: the mortality of nosocomial MRSA bloodstream infection

✂: preventing

👍: improving

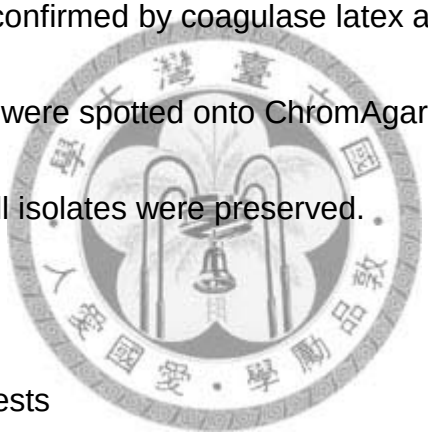
The study framework was illustrated above (Figure 3 – 1). Prior study on pathogenesis of MRSA infection demonstrated that carriage of MRSA usually developed before infection caused by MRSA.[5] Our study was aimed on the primary and secondary prevention for CA-MRSA infection among adult population, including identification of risk factors for carriage of CA-MRSA and mortality of nosocomial bloodstream infection caused by MRSA (focus on the impact of CA-MRSA on nosocomial MRSA bloodstream infection). After identification of these risk factors, we then can conduct interventions to prevent

carriage of CA-MRSA and improve the outcome of MRSA infection.

2 Microbiologic studies

2.1 Bacterial culture and identification of MRSA

Each swab was plated onto a sheep blood agar (SBA) plate. All plates were incubated at 35℃ ambient air for 48 hours. Isolates suspected to be *S. aureus* from SBA were first checked by catalase and Gram-stain if necessary, and all *S. aureus* were confirmed by coagulase latex agglutination. *S. aureus* isolates from SBA plate were spotted onto ChromAgar MRSA to check for methicillin-resistance. All isolates were preserved.



2.2 Drug susceptibility tests

All isolates were tested for their minimum inhibitory concentration (MIC) levels of gentamicin, clindamycin, erythromycin, ciprofloxacin, minocycline, rifampin, trimethoprim/sulfamethoxazole, and vancomycin using agar dilution method proposed by the National Committee for Clinical Laboratory Standards (NCCLS).[114] In brief, a Steers' replicator was used to apply 10^4 CFUs of bacteria onto Mueller-Hinton agar containing serial two-fold dilutions of each antimicrobial agent (0.03 – 256 μg/mL). The agar plates were incubated at 35

℃ for 18 hours (24 hours for vancomycin) before reading. The MIC was determined as the lowest concentration of antimicrobial agents completely inhibiting the growth of bacteria. *S. aureus* American Type Culture Collection (ATCC) 29213 was used as internal control in each run of the test. The breakpoints used for reading as susceptible were as defined by the Clinical and Laboratory Standards Institute (CLSI).[115]

2.3 Multi-locus sequence typing (MLST)

2.3.1 PCR and neucleotide sequence used for MLST

The chromosomal DNA were prepared using the method provided by previous reports.[116, 117] PCR amplification was performed using 1 unit AmpliTaq (Perkin-Elmer Cetus, Foster City, Calif.) in 50 μl of reaction mixture (10mM Tris-HCl [pH 8.3], 50 mM KCl, 0.001% [wt/vol] gelatin, 50% [vol/vol] glycerol, 1.5 mM $MgCl_2$, 200 mM each deoxynucleoside triphosphatge, 1.0 mM each primer, and template DNA). The reaction was carried out by using a Gene Amp PCR system 9600 (Perkin-Elmer). Thermal cycling was set at 30 cycles (30 s for denaturation at 94℃, 1 min for annealing at 50℃, and 2 min for elongation at 72℃)

All PCR products were further sequenced using a 377 automated

fluorescent DNA sequencing system (Perkin-Elmer, Foster City, Calif.) to compare the nucleotide homology with the published sequence in GenBank.

2.3.2 PCR primers used for MLST

The primers used for the detection of fragments of 7 housekeeping genes, including *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*, in *S. aureus* were list below:[26]

Gene	Primer	Sequence (5'-3')
Carbamate kinase (*arcC*)	*arcC*-Up	TTGATTCACCAGCGCGTATTGTC
	arcC-Dn	AGGTATCTGCTTCAATCAGCG
Shikimate dehydrogenase (*aroE*)	*aroE*-Up	ATCGGAAATCCTATTTCACATTC
	aroE-Dn	GGTGTTGTATTAATAACGATATC
Glycerol kinase (*glpF*)	*glpF*-Up	CTAGGAACTGCAATCTTAATCC
	glpF-Dn	TGGTAAAATCGCATGTCCAATTC
Guanylate kinase (*gmk*)	*gmk*-Up	ATCGTTTTATCGGGACCATC
	gmk-Dn	TCATTAACTACAACGTAATCGTA
Phosphate acetyltransferase (*pta*)	*pta*-Up	GTTAAAATCGTATTACCTGAAGG
	pta-Dn	GACCCTTTTGTTGAAAAGCTTAA
Triosephosphate isomerase (*tpi*)	*tpi*-Up	TCGTTCATTCTGAACGTCGTGAA
	tpi-Dn	TTTGCACCTTCTAACAATTGTAC
Acetyl coenzyme A acetyltransferase (*yqiL*)		
	yqiL-Up	CAGCATACAGGACACCTATTGGC
	yqiL-Dn	CGTTGAGGAATCGATACTGGAAC

2.3.3 Determination of the allelic profile of MLST

All sequences of 7-paired PCR products of every MRSA isolates were compared with existing database using internet software on internet website (http://saureus.mlst.net). Allelic profile of every isolate was then determined and a ST was assigned.

2.4 SCC*mec* element typing

The primers used to undergo PCR for determining the SCC*mec* element types (I, II, III, IV, and V) and the multiplex PCR methods were according to those proposed by Zhang et al.[83] The primers were listed as follows.

Primer	Oligonucleotide sequence (5' – 3')	Specificity
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	SCC*mec* I
Type I-R	GTTCTCTCATAGTATGACGTCC	
Type II-F	CGTTGAAGATGATGAAGCG	SCC*mec* II
Type II-R	CGAAATCAATGGTTAATGGACC	
Type III-F	CCATATTGTGTACGATGCG	SCC*mec* III
Type III-R	CCTTAGTTGTCGTAACAGATCG	
Type IVa-F	GCCTTATTCGAAGAAACCG	SCC*mec* IVa
Type IVa-R	CTACTCTTCTGAAAAGCGTCG	
Type IVb-F	TCTGGAATTACTTCAGCTGC	SCC*mec* IVb
Type IVb-R	AAACAATATTGCTCTCCCTC	
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	SCC*mec* IVc
Type IVc-R	TTGGTATGAGGTATTGCTGG	
Type IVd-F5	CTCAAAATACGGACCCCAATACA	SCC*mec* IVd
Type IVd-R6	TGCTCCAGTAATTGCTAAAG	
Type V-F	GAACATTGTTACTTAAATGAGCG	SCC*mec* V
Type V-R	TGAAAGTTGTACCCTTGACACC	
MecA147-F	GTG AAG ATA TAC CAA GTG ATT	*mecA*
MecA147-R	ATG CGC TAT AGA TTG AAA GGA T	
mecI-F	CCCTTTTTATACAATCTCGTT	Class A *mec*
mecI-R	ATATCATCTGCAGAATGGG	
IS1272-F	TATTTTTGGGTTTCACTCGG	Class B *mec*
mecR1-R	CTCCACGTTAATTCCATTAATACC	
ccrAB-β2	ATTGCCTTGATAATAGCCITCT	
ccrAB-α2	AACCTATATCATCAATCAGTACGT	Type 1 *ccr*
ccrAB-α3	TAAAGGCATCAATGCACAAACACT	Type 2 *ccr*
ccrAB-α4	AGCTCAAAAGCAAGCAATAGAAT	Type 3 *ccr*
ccrC-F	ATGAATTCAAAGAGCATGGC	Type 5 *ccr*
ccrC-R	GATTTAGAATTGTCGTGATTGC	

The PCR procedures were described in brief as follows. An aliquot of 2 μl of DNA suspension was added to 23 μl of PCR mixture containing 50 mM KCl, 20

mM Tris-HCl (pH 8.4), 2.5 mM MgCl2, 0.2 mM of each deoxynucleoside triphosphate, the respective primers, and 1.0 unit of Platinum *Taq* DNA polymerase (Invitrogen Inc., Carlsbad, CA). The amplification was performed in a Gene Amp PCR system 9600 (Perkin-Elmer) beginning with an initial denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, ending with a final extension step at 72°C for 10 min and followed by a hold at 4°C.

2.5 PCR for PVL gene

The primers used to undergo PCR for detection of PVL gene were according to those proposed by Lina et al.[84]

For *luk-PV-1*, 5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'

For *luk-PV-2*, 5'-GCATCAASTGTATTGGATAGCAAAAGC-3'

The PCR cycle was 30 s of denaturation at 94℃, 30 s of annealing at 55℃, and 1 min of extension at 72℃. After 30 cycles, the amplicons were resolved by electrophoresis through 1.5% agarose gels. This procedure was followed by ethidium bromide staining and analysis. Amplicon were subjected to DNA sequencing as described above. *S. aureus* ATCC 49775 was used as positive

amplification controls for all PCR runs, whereas pure water was used as a negative amplification control.

Since the CA-MRSA isolates in Taiwan were not necessary to carry PVL gene, MRSA isolates with type IV or V SCC*mec* element were considered as CA-MRSA in the following studies.[106, 111–113]

3. Three studies

3.1 Prevalence and risk factors of nasal carriage of CA-MRSA among healthy adults

3.1.1 Study population and collection of specimen

From October 1, 2007 to December 31, 2007, all adults attending a mandatory health examination as part of workplace health promotion at Taipei Cathay General Hospital (KTC), Wanfang Hospital (WFH), and Far Eastern Memorial Hospital (FEMH) and having signed the informed consent were eligible for enrollment. Every person enrolled in this study was then taken a nasal swab by a well-trained study assistant. There were 3098 people enrolled in total, including 991 in KTC, 1484 in WFH, and 623 in FEMH. The swabs were sent back to the central laboratory located in NTUH for primary cultures within six hours.

3.1.2 Study variables

A standardized questionnaire was used to collect information for analyzing the potential independent variables (risk factors) of CA-MRSA nasal carriage. The questionnaire was appended as Appendix 1.

- Dependent variable in this study: The dependent variable was multinominal and classified into three groups, including those with nasal carriage of CA-MRSA, those with nasal carriage of MSSA, and those without carriage of *S. aureus*. The prevalence of CA-MRSA nasal carriage among healthy adults (age > 18 years) was estimated.
- Potential independent variables:
    a. Demographic variables: age, gender, education level, occupation, marriage status, residential, number of household members, having household members being healthcare workers, having household members aged less than 7 years, having bed-ridden household member, and economic status measured by family income.
    b. Vairables of life style: These included smoking or not, and taking shower every day or not.
    c. Variables of healthcare- or health-associated facilities:

hospitalization within prior one year, caring for inpatient within one year, visiting outpatient clinic within on year, usage of antibiotics within prior one year.

d. Variables of activities: having tattoo and/or acupuncture within one year, and attending to the public places (including hot spring bath, Solus Par Aqua, swimming pool, sauna bath, gymnasium, dancing saloon)

e. Variables of co-morbidity: the presence of chronic diseases (including allergic rhinitis, asthma, emphysema, diabetes mellitus, hypertension, coronary artery disease, heart failure, stroke, liver cirrhosis, end-staged renal disease, cancer, autoimmune diseases) or not, and history of skin and soft-tissue infection within prior one year.

Among them, age and number of household members were treated as continuous variables; the others as categorical variables.

3.2 Prevalence and risk factors of carriage of CA-MRSA among ICU adult patients

3.2.1 Patients and collection of specimen

From September 1, 2008 to September 30, 2009 (but the study was interrupted from March 1 to March 31, 2009), all patients admitted to the medical ICU (MICU) and coronary care unit (CCU) at the FEMH were enrolled in this study. The surveillance cultures were taken on all patient in MICU and CCU as follows: 1) all patients in these two ICUs on the first day of study period had surveillance cultures taken on day 1; 2) patients who were newly admitted to ICUs had surveillance cultures taken within 24 hours of admission; 3) for all patients, surveillance cultures were taken every three days and on the day they left the ICUs; 4) the culture sites of every surveillance culture included the nostril,[119 – 121] throat (or sputum if intubated),[122, 123] axillae,[124] and inguinal area[124]. All swabs were sent back to the central laboratory in NTUH within six hours for bacterial culture and subsequent microbiologic studies. A total of 1906 patients, 1207 in CCU and 699 in MICU, were enrolled in this study. In addition, although patients who had carried one strain of MRSA were still at risk to acquire another strain of MRSA theoretically, however, these patients might have different clinical features compared to others. Therefore, only the first instance when MRSA was found was considered in the following analysis.

3.2.2 Study variables

A standardized case report form was used to collect the potential independent variables (risk factors) for MRSA carriage. The case report form was appended as Appendix 2.

- Dependent variables in this study: The depdent variable was multinominal and classified into three groups, including patients newly acquiring CA-MRSA during their ICU stays, patients newly acquiring HA-MRSA during their ICU stays, and patients without MRSA carriage.
- Potential independent variables:
    a. Demographic variables: age, gender, and occupation.
    b. Variable of life style: smoking or not.
    c. Variables of healthcare facilities, devices and medication: length of ICU stay, steroid usage, recent operation, usage of central venous catheter, usage of arterial line (A-line), usage of nasogastric (NG) tube, usage of Foley catheter, intubation, and antimicrobial usage.[125] The antimicrobial agents were classified into 17 groups: group 1, penicillins without anti-pseudomonal effect and not combined with β-lactamase inhibitors; group 2, anti-pseudomonal penicillins; group 3, penicillins combined with

β-lactamase inhibitors; group , first-generation cephalosporins; group 5, second-generation cephalosporins; group 6, third-generation cephalosporins without anti-pseudomonal effect; group 7, third-generation cephalosporins with anti-pseudomonal effect; group 8, fourth-generation cephalosporins; group 9, carbapenems; group 10, monobactam; group 11, glycopeptides; group 12, anti-anaerobic agents and antibiotics with effect against atypical pathogens; group 13, aminoglycosides; group 14, anti-fungals; group 15, fluoroquinolones; group 16, colistin; group 17, tigecycline.

d. Variables of co-morbidity: the Acute Physiology and Chronic Health Evaluation (APACHE) II score (dichotomized into two strata: equal to or over 17 and less than 17) upon admission to ICUs and underlying diseases,[125] including cardiovascular diseases, respiratory diseases, heptobiliary diseases, genitobiliary diseases, gastroenterologic diseases, mucocutaneous diseases, neurovascular diseases, endocrinologic diseases, autoimmune diseases, and malignancies.

Among them, age and length of ICU stay were treated as continuous variables; the others as categorical variables.

3.3 Mortality of nosocomial MRSA bloodstream infection

3.3.1 Patients

From January 1, 2006 to December 31, 2008, all adult patients (over 18 of age) admitted to NTUH with nosocomial MRSA bloodstream infection were included as the source subjects of this study. The nosocomial MRSA bloodstream infection was defined as MRSA isolated from two sets of blood culture taken from two different sites 48 hours after admission. When a patient had two successive isolates of MRSA from his or her blood, and the time interval between these two episodes was longer than 3 months, he or she was considered as having two episodes of MRSA bloodstream infection. However, because of the possibility that patients with more than one episode of MRSA bloodstream infection would have different demographic and clinical features compared to those with only one episode of MRSA bloodstream infection, only first episode of MRSA bacteremia was enrolled for outcome analysis. The blood isolates were obtained from the Department of Laboratory Medicine at NTUH and then sent to the central laboratory of this study. There were 308

episodes was enrolled.

3.3.2 Study variables

A standardized case report form was used to collect the potential independent variables (risk factors) for Day 14 and/or Day 30 all-cause mortality of nosocomial MRSA bloodstream infection. The case report form was appended as Appendex 3.

- Dependent variables in this study: all-casue Day 14 and Day 30 mortality.
- Potential independent variables:
    a. Demographic variables: age, gender, and sites (general ward or ICU) where the bloodstream infection developed.
    b. Variables related MRSA bloodstream infection: primary focus of MRSA bloodstream infection and severity of infection (shock or not).[126]
    c. Variables of healthcare-associated facilities, devices, and medication: presence of prosthesis while onset of MRSA bloodstream infection, and effective treatment within 48 hours after MRSA bloodstream infection.
    d. Variables of comorbidity: underlying diseases (including

cardiovascular diseases, respiratory diseases, heptobiliary diseases, genitobiliary diseases, gastroenterologic diseases, mucocutaneous diseases, neurovascular diseases, endocrinologic diseases, autoimmune diseases, and malignancies), Charlson comorbidity index,[127] immune status[125] while onset of MRSA bloodstream infection

e. Variables of laboratory data: serum albumin level, leukocyte count, platelet count, serum C-reactive protein (CRP) level, serum creatinine level, serum alanine aminotransferase level, serum lactate dehydrogenase level while onset of MRSA bloodstream infection. All the variables related to laboratory examinations except CRP level were further dichotomized into categorical variables with two strata (normal or abnormal) using the criteria provided by NTUH.[125]

Among them, age, Charlson comorbidity index, and CRP level were treated as continuous variables; the others as categorical variables.

4. Statistics

Continuous variables were described as mean ± standard deviation (SD) and compared using Student's *t* test, or described as median as well as range and compared with Wilcoxon Rank-Sum test if their distributions were not normal. Categorical variables were compared with a chi-square test or Fisher exact test if the expected number was less than 5. The 95% confident intervals (C.I.) of prevalence rates were estimated using normal theory test if the expected number was equal to or more than 5 ($N \times P \geq 5$), or exact method if the expected number was less than 5 ($N \times P < 5$). The 95% C.I.s of incidencs were estimated using a Poisson distribution. In the study considering CA-MRSA carriage among healthy adults, predictors for carriage of CA-MRSA were identified using a polytomous logistic regression model by comparing the characteristics of people who carried CA-MRSA with those who were negative for *S. aureus*, and those who were positive for *S. aureus* but not MRSA. In the study considering new acquirement of CA-MRSA carriage among adult inpatients, also a polytomous logistic regression model by comparing the characteristics of patients who newly acquired CA-MRSA with those who newly acquired HA-MRSA, and those who were negative for *S. aureus* was used to identified the predictors. In the study of outcome analysis of nosocomial MRSA bloodstream infection, risk factors for Day 14 and Day 30 all-cause mortality

were determed by binary logistic regression models. All parameters were initially tested by univariate analysis and those with a p value less than 0.2 were used for multivariate analysis. In these regression models, parameters with colinearity, which was determined by clinical judgement first and then by using the value of variance inflation greater than 10 determined by a linear regression model using the VIF option in Statistics Analysis System (SAS) software, were not considered in the final model simultaneously.[118] A stepwise model comparison and selection by calculating the -2 log likelihood were used to determine the final model of multivariate analysis. All tests were two-tailed and a p value less than 0.05 was considered statistically significant. All the statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, N.C., U.S.A.).

Chapter 4 Results

1 Prevalence and risk factors of nasal arriage of CA-MRSA among healthy adults

1.1 Prevalence

During the three-month study period, there were 3098 people enrolled. Among them, 687 people were found to carry *S. aureus*. A total of 120 of these 687 people carried MRSA, and 567 carried MSSA. The comparisons of demographics and other parameters of the enrolled people are shown in Table 4 – 1. There were statistically significant differences between these three groups in the parameters of gender, educational level, having any household member being a healthcare worker, having any household member less than 7 years old, smoking, and the use of antibiotics within the past year. Based on post hoc analysis, we found that people with MRSA were more likely to have household members who were less than 7 years old (both $p<0.0001$) and more likely to have used antibiotics during past year ($p=0.0012$ and 0.0004, respectively) compared to the other two groups, as well as less likely to be smokers compared to those without *S. aurues* colonization ($p=0.0077$).

Among the 120 MRSA isolates from the 120 people (henceforth “index people”), 100 were classified as ST59, 10 as ST508, five as ST89, two as

ST239, 1 as ST6, and 2 as untypable. Of the 100 isolates of ST59, 65 carried the type IV SCC*mec* element and 35 carried the type V SCC*mec* element. Of the 65 ST59-IV MRSA isolates, only 10 (15.4%) were positive for the PVL gene. All 35 of the ST59-V isolates were positive for the PVL gene. All isolates of ST6 and ST508 carried the type IV SCC*mec* element, all isolates of ST89 carried the type II SCC*mec* element, and both isolates of ST239 carried the type III SCC*mec* element (Table 4 – 2). All isolates, except two of ST508, belonged to ST6, ST89, ST239, and ST508 were negative for the PVL gene. If taking those isolates carrying type IV and V SCC*mec* elements into consideration as CA-MRSA strains, the prevalence of CA-MRSA nasal carriage among healthy adults in Taiwan was 3.6% (111/3098, 95% C.I.: 2.9% – 4.3%).

1.2 Risk factors for CA-MRSA nasal carriage

Univariate analysis indicated female gender, having healthcare workers in the household, having household members less than 7 years old, non-smoking, and use of antibiotics during the past year were risk factors for CA-MRSA nasal carriage (Table 4 – 3). Using multivariate analysis, having household members less than 7 years old, and use of antibiotics during the past year were independent risk factors for CA-MRSA nasal carriage (odds

ratio: 2.14, and 1.91, respectively; 95% C.I.: 1.15 – 3.97, and 1.26 – 2.90, respectively; P = 0.0001, and 0.0027, respectively) compared to those without carriage of *S. aureus*. Smoking was found to be a significant factor inhibiting nasal carriage of CA-MRSA (odds ration: 0.49; 95% C.I.: 0.32 – 0.74; P = 0.0201) compared to those without carriage of *S. aureus*. However, having household members less than 7 years old, and use of antibiotics during the past year were the only two independent risk factors for MRSA colonization (odds ratio: 2.83 and 1.99; 95% C.I.: 1.46 – 5.48 and 1.26 – 3.15; P <0.0001 and = 0.0040) compared to those with carriage of MSSA (Table 4 – 4).

Table 4 – 5 shows the drug susceptibilities of all 111 CA-MRSA isolates stratified by MLST types. The overall susceptibilities were 21.6% for clindamycin, 16.2% for erythromycin, 100% for trimethoprim/sulfamethoxazole, 75.7% for gentamicin, 99.1% for minocycline, 100% for ciprofloxacin, 100% for rifampin, and 100% for vancomcyin.

Table 4 – 1. Characteristics of people with MRSA, MSSA, and no *S. aureus* colonization (n=3098).

Parameters	MRSA (n=120)	MSSA (n=567)	No_C (n=2411)	p value
Age (mean±SD)	38.1±12.7	39.5±11.9	39.9±11.6	0.2177
Gender*				0.0187
Male	51 (42.0%)	275 (48.8%)	1013 (42.3%)	
Female	69 (58.0%)	288 (51.2%)	1381 (57.7%)	
Education*				
Under elementary school	3 (2.5%)	3 (0.5%)	22 (0.9%)	0.0287
Elementary school	8 (6.8%)	19 (3.4%)	68 (2.9%)	
Junior high school	4 (3.4%)	11 (2.0%)	78 (3.3%)	
Senior high school	19 (16.1%)	74 (13.3%)	375 (15.9%)	
University	59 (50.0%)	324 (58.2%)	1380 (58.5%)	
Graduate or beyond	25 (21.2%)	126 (22.6%)	437 (18.5%)	
Status of marriage*				
Married	87 (75.0%)	359 (65.5%)	1568 (66.9%)	0.3079
Divorced	1 (0.9%)	11 (2.0%)	56 (2.4%)	
Unmarried	28 (24.1%)	178 (32.5%)	720 (30.7%)	
Being a HCW*				0.1967
Yes	13 (13.1%)	40 (8.1%)	212 (10.3%)	
No	86 (86.9%)	453 (91.9%)	1843 (89.7%)	
Living in dormitories*				0.7715
Yes	1 (1.2%)	10 (2.0%)	37 (2.2%)	
No	85 (98.8%)	406 (98.0%)	1674 (97.8%)	

Table 4 – 1. continued

Parameters	MRSA (n=120)	MSSA (n=567)	No_C (n=2411)	p value
No. of household members	4.0±1.6	3.7±1.5	3.7±1.6	0.0665
Having household members being				0.0087
HCWs*				
Yes	10 (8.6%)	54 (9.7%)	144 (6.1%)	
No	106 (91.4%)	504 (90.3%)	2208 (93.9%)	
Having household members less				<0.0001
than 7 years old*				
Yes	52 (44.4%)	121 (21.7%)	610 (25.8%)	
No	65 (55.6%)	437 (78.3%)	1758 (74.2%)	
Presence of household members				0.7526
being bed-ridden*				
Yes	4 (3.4%)	18 (3.2%)	64 (2.7%)	
No	114 (96.6%)	541 (96.8%)	2298 (97.3%)	
Chronically ill*				0.4702
Yes	42 (36.5%)	180 (32.7%)	741 (31.5%)	
No	73 (63.5%)	370 (67.3%)	1614 (68.5%)	
Smoking*				<0.0001
Yes	14 (11.8%)	76 (13.5%)	504 (21.2%)	
No	105 (88.2%)	485 (86.5%)	1876 (78.8%)	
Hospitalization*				0.3672
Yes	9 (7.6%)	25 (4.5%)	128 (5.4%)	
No	110 (92.4%)	534 (95.5%)	2255 (94.6%)	

Table 4 – 1. continued

Parameters	MRSA (n=120)	MSSA (n=567)	No_C (n=2411)	p value
Caring for inpatient*				0.6599
Yes	22 (19.1%)	90 (16.2%)	416 (17.4%)	
No	93 (80.9%)	465 (83.8%)	1951 (82.4%)	
Visiting outpatient clinics*				0.6045
Yes	78 (67.2%)	364 (64.8%)	1587 (67.0%)	
No	38 (32.8%)	198 (35.2%)	783 (33.0%)	
Using antibiotics*				0.0020
Yes	35 (29.9%)	95 (17.1%)	409 (17.2%)	
No	82 (70.1%)	461 (82.9%)	1963 (82.8%)	
Tattoo or acupuncture or using parenteral drug or dialysis*				0.7852
Yes	2 (1.7%)	16 (2.8%)	64 (2.7%)	
No	115 (98.3%)	546 (97.2%)	2309 (97.3%)	
Skin or soft-tissue injury*				0.8420
Yes	55 (47.8%)	282 (50.8%)	1186 (50.4%)	
No	60 (52.2%)	273 (49.2%)	1165 (49.6%)	
Shower everyday*				0.3528
Yes	113 (96.6%)	539 (96.3%)	2314 (97.4%)	
No	4 (3.4%)	21 (3.7%)	62 (2.6%)	
Visiting public amusement places*				0.1884
Yes	68 (57.6%)	322 (57.5%)	1453 (61.4%)	
No	50 (42.4%)	238 (42.5%)	913 (38.6%)	

Table 4 – 1. continued

Parameters	MRSA (n=120)	MSSA (n=567)	No_C (n=2411)	p value
Family income*				
Less than 20,000 NTD	1 (2.4%)	1 (0.6%)	17 (2.5%)	0.4533
20,000 – 50,000 NTD	3 (7.1%)	28 (16.3%)	108 (15.8%)	
50,000 – 100,000 NTD	20 (47.6%)	64 (37.2%)	244 (35.7%)	
100,000 – 200,000 NTD	8 (19.0%)	38 (22.1%)	123 (18.0%)	
200,000 – 300,000 NTD	3 (7.1%)	5 (2.9%)	31 (4.5%)	
Over 300,000 NTD	7 (16.7%)	36 (20.9%)	160 (23.4%)	

Abbreviations: No_C, no *S. aureus* colonization; SD, standard deviation; M, male; F, female; HCWs, healthcare workers; NTD, new Taiwan dollar

*There is missing data for some parameters, including 21 in “sex”, 63 in “education”, 95 in “being a HCW”, 885 in “living in dormitories”, 72 in “presence of household members being HCWs”, 55 in “presence of household members aged under 7”, 59 in “presence of household members being bed-ridden”, 78 in “chronically ill”, 38 in “smoking”, 37 in “hospitalization”, 61 in “caring for inpatients”, 50 in “visiting outpatient clinics”, 53 in “using antibiotics”, 48 in “Tattoo and/or acupuncture and/or using parenteral drug and/or dialysis”, 77 in “skin or soft-tissue injury”, 47 in “shower everyday”, 54 in “Visiting public amusement places”, and 2201 in “family income”.

Table 4 – 2. MLST types and SCC*mec* elements in the 120 MRSA isolates

MSLT type	SCC*mec* type				
	II	III	IV	V	Subtotal
ST6	0	0	1	0	1
ST59	0	0	65	35	100
ST89	5	0	0	0	5
ST239	0	2	0	0	2
ST508	0	0	10	0	10
Untypable					2
Total	5	2	76	35	120

Table 4 – 3. Risk factors for nasal carriage of CA-MRSA by univariate analysis using polytomous logistic regression.

Parameter	CA-MRSA vs No_C		CA-MRSA v.s. MSSA		P value of overall model
	OR	P value	OR	P value	
Age	0.99	0.0961	0.99	0.2151	0.2175
Gender	1.01	0.9679	0.77	0.2071	0.0189
Education degree (using "under elementary school" as baseline)					
Elementary school	3.35	0.0014	0.66	0.3959	<.0001
Junior high school	0.586	0.1955	0.44	0.1029	0.2639
Senior high school	1.64	0.2807	3.55	0.0181	0.0145
University	3.19	0.0022	0.45	0.1263	<.0001
Graduate or beyond	1.20	0.8029	0.22	0.0580	<.0001
Marital status (using "unmarried" as baseline)					
Married	1.43	0.1092	1.54	0.0669	0.1852
Divorced	0.46	0.4485	0.58	0.6063	0.6133
Being a HCW	1.31	0.3724	1.72	0.1141	0.1991
Living in dormitories	0.53	0.5362	0.48	0.4839	0.7774
No. of household members	1.12	0.0388	1.15	0.0196	0.0653
Presence of household members being HCWs	1.47	0.2801	0.88	0.7240	0.0094
Presence of household members ≦7 years old	2.31	<.0001	2.89	<.0001	<.0001
Presence of bedridden household members	1.26	0.6595	1.05	0.9247	0.7533

Table 4 – 3. Continued

Parameter	CA-MRSA vs No_C		CA-MRSA v.s. MSSA		P value of overall model
	OR	P value	OR	P value	
Chronically ill	1.25	0.2560	1.18	0.4329	0.4711
Smoking	0.50	0.0153	0.85	0.6026	<.0001
Hospitalization within the past year	1.44	0.3077	1.75	0.1655	0.3711
Cared for inpatients within the past year	1.1	0.6693	1.22	0.4464	0.6602
Visited outpatient clinics within the past year	1.01	0.9502	1.12	0.6108	0.6047
Used antibiotics within the past year	2.05	0.0006	2.07	0.0016	0.0025
Tattoo and/or acupuncture and/or using parenteral drugs and/or dialysis	0.63	0.5199	0.59	0.4907	0.7885
Skin or soft-tissue injury within the past year	0.90	0.5832	0.89	0.5602	0.8420
Shower everyday	0.77	0.6167	1.107	0.8629	0.3563
Visited public amusement places within the past year	0.85	0.4106	1.01	0.9797	0.1888
Family income (NTD)					
20,000-50,000	0.47	0.5262	0.11	0.1467	0.2864
50,000-100,000	1.39	0.7531	0.32	0.4183	0.3454

Table 4 – 3. Continued

Parameter	CA-MRSA vs No_C		CA-MRSA v.s. MSSA		P value of overall model
	OR	P value	OR	P value	
100,000-200,000	1.11	0.9267	0.21	0.2881	0.2842
200,000-300,000	1.65	0.6766	0.60	0.7483	0.6324
Over 300,000	0.74	0.7876	0.19	0.2663	0.4139

Abbreviations: No_C, no *S. aureus* colonization; HCWs, healthcare workers; NTD, new Taiwan dollar.

Table 4 – 4. Risk factors for CA-MRSA nasal carriage compared to MSSA carriage and no *S. aureus* carriage by multivariate analysis using polytomous logistic regression.

Parameters	CA-MRSA v.s. No_C		CA-MRSA v.s. MSSA		p value of overall model
	Odds ratio (95% confidence interval)	p value of co-efficient	Odds ratio (95% confidence interval)	p value of co-efficient	
Presence of household members aged ≤ 7	2.14 (1.15 – 3.97)	0.0001	2.83 (1.46 – 5.48)	<0.0001	<0.0001
Smoking	0.49 (0.32 – 0.74)	0.0201	1.02 (0.65 – 1.62)	0.9440	<0.0001
Using antibiotics within past 1 year	1.91 (1.27 – 2.90)	0.0027	1.99 (1.26 – 3.15)	0.0040	0.0091

Abbreviation: No_C, no *S. aureus* colonization.

Table 4 – 5. Drug susceptibilities of the 111 CA-MRSA isolates with stratification by MLST type.

MLST types (no. of isolates)	CM	ERM	TXT	GM	MIN	CIP	RIF	VAN
ST6 (1)	100%	100%	100%	100%	100%	100%	100%	100%
ST59 (100)	14.0%	12.0%	100%	74.0%	99%	100%	100%	100%
ST508 (10)	90.0%	50.0%	100%	90.0%	100%	100%	100%	100%
Overall (111)	21.6%	16.2%	100%	75.7%	99.1%	100%	100%	100%

Abbreviations: CM, clindamycin; ERM, erythromycin; TXT, trimethoprim/sulfamethoxazole; GM, gentamicin; MIN, minocycline; CIP, ciprofloxacin; RIF, rifampin; VAN, vancomycin.

2 Prevalence and risk factors of carriage of CA-MRSA among ICU adult patients

2.1 Prevalence and incidence of new acquirement of CA-MRSA carriage

During the study period, there were 1906 patients screened in total. Among these 1906 patients, 203 patients were found to have carried MRSA before they were admitted to ICU. Eighty-one patients were found to have newly acquired MRSA during their stay in ICUs. The overall patient-days at risk for acquiring MRSA while staying in ICU were 10320 patient-days. Therefore, the incidence of acquiring MRSA during ICU stay was 7.9 per 1000 patient-days (95% C.I., 6.3 – 9.8 per 1000 patient-days). The overall MRSA prevalence of ICU patients was 14.9% (95% C.I.: 13.3% – 16.5%).

Of the 284 MRSA isolates from the 284 patients (188 isolates from MICU, and 96 isolates from CCU), 148 belonged to ST59 carrying type IV or V SCC*mec* elements, 27 belonged to ST5 carrying type II SCC*mec* element, 102 belonged to ST239 carrying type III SCC*mec* element, and 7 were untypable carrying type II or III SCC*mec* elements. Therefore, CA-MRSA isolates accounted for 52.8% of all MRSA isolated from ICU in the present study. Of the 188 MRSA isolates from MICU, 78 belonged to CA-MRSA isolates. Of the 96 isolates from CCU, 70 belonged to CA-MRSA isolates. The distribution is

significantly different (P<0.0001). Among the 81 patients who newly acquired MRSA during their ICU stay, there were 31 patients whose MRSA isolates belonged to ST59 and carried type IV (11 isolates, 4 positive for PVL gene) or V (20 isolates, all positive for PVL gene) SCC*mec* elements. These 31 MRSA isolates were assumed to be CA-MRSA. The other 50 MRSA isolates carried type II or III SCC*mec* elements, and were assumed to be HA-MRSA. In this way, the incidence of acquiring CA-MRSA during ICU stay was 3.0 (95% C.I.: 2.1 – 4.3) per 1000 patient-days.

2.2 Risk factors for new acquirement of CA-MRSA carriage during ICU stay

The epidemiological and clinical data of the 31 patients who newly acquired CA-MRSA during ICU stay, the 50 patients who newly acquired HA-MRSA during ICU stay, and the 1622 patients who did not carry MRSA were listed in Table 4 – 6. Using post hoc analysis, patients without MRSA colonization had shorter duration of ICU stay and shorter duration at risk for acquiring MRSA in ICU, lower proportion with APACHE II score > 17, underlying respiratory diseases, underlying genitourinary tract diseases, using central venous catheter, using arterial line, using NG tube, using Foley catheter, being intubation, and prior usage of group 5, 6, 7, 8, as well as 14 antimicrobial

agents, while a higher proportion to use aspirin comparing to patients newly acquiring CA-MRSA. Patients acquiring HA-MRSA during ICU stay had lower proportion of underlying cardiovascular diseases, but higher proportion of underlying hepatobiliary diseases, underlying malignancies, with prior usage of group 9, 12, 13, 15, and 16 antimicrobial agents comparing to patients newly acquiring CA-MRSA.

Univariate analysis to identify the risk factors revealed that a high APACHE II score (> 17), presence of underlying respiratory diseases, nasogastric (NG) tube, endotracheal tube, prior usage of anti-pseudomonal penicillins, third-generation cephalosporins without anti-pseudomonal effect, and anti-fungal agents were significantly associated with new acquirement of CA-MRSA carriage during ICU stay while compared to those without MRSA carriage. In the other way, prior usage of carbapenems, presence of Foley catheter, and underlying cardiovascular diseases were significantly associated with new acquirement of CA-MRSA carriage during ICU stay while compared to those who newly acquired HA-MRSA carriage during ICU stay (Table 4 – 7).

Multivariate analysis showed that presence of NG tube, prior usage of anti-pseudomonal penicillins and anti-fungals (odds ratio: 3.53, 3.09, and 3.45, respectively; 95% C.I.: 1.16 – 10.75, 1.45 – 6.58, and 1.11 – 10.74,

respectively; P = 0.0262, 0.0035, and 0.0330, respectively) were independent risk factors for new acquirement of CA-MRSA carriage during ICU stay while compared to those without MRSA carriage. However, prior usage of carbapenems was a protective factor (odds ratio, 0.08; 95% C.I., 0.01 – 0.72; P = 0.0240) against new acquirement of CA-MRSA carriage during ICU stay while compared to those who newly acquired HA-MRSA carriage during ICU stay (Table 4 – 8).

Table 4 – 6. The demographic and clinical data of the 81 patients who newly acquired MRSA and the 1622 patients without MRSA during ICU stay

Parameters[a]	CA-MRSA (N=31)	HA-MRSA (N=50)	No_C (N=1622)	P vaule
Age	70.5±12.2	66.9±18.6	64.9±50.5	0.7927
Gender				0.7760
Male	19	33	1090	
Female	12	17	532	
Duration of ICU stay	12.0±7.5	14.4±10.3	6.0±6.8	<0.0001
Time at risk for acquiring MRSA	7.3±6.8	8.7±6.4	6.0±6.8	0.0128
APACHE II > 17	20 (64.5%)	36 (73.5%)	510 (31.8%)	<0.0001
Smoking	8 (25.8%)	22 (44.0%)	613 (37.8%)	0.2574
Cardiovascular dz	25 (80.6%)	27 (54.0%)	1172 (72.3%)	0.0101
Respiratory dz	20 (64.5%)	40 (80.0%)	536 (33.0%)	<0.0001
Hepatobiliary dz	2 (6.5%)	12 (24.0%)	87 (5.4%)	<0.0001
Genitourinary dz	14 (45.2%)	19 (38.0%)	452 (27.9%)	0.0341
GI tract dz	5 (16.1%)	10 (20.0%)	252 (15.5%)	0.6922
Mucocutaneous dz	2 (6.5%)	1 (2.0%)	54 (3.3%)	0.5473
Neurovascular dz	8 (25.8%)	9 (18.0%)	214 (13.2%)	0.0824
Endocrinologic dz	17 (54.8%)	20 (40.0%)	579 (35.7%)	0.0760
Recent operation	11 (35.5%)	10 (20.0%)	832 (51.3%)	<0.0001
Usage of CVC	20 (64.5%)	38 (76.0%)	761 (46.9%)	<0.0001
Usage of A-line	31 (100%)	48 (96.0%)	1275 (78.6%)	0.0002
Usage of NG tube	27 (87.1%)	46 (92.0%)	870 (53.6%)	<0.0001
Usage of Foley Ca.	23 (74.2%)	46 (92.0%)	1062 (65.5%)	0.0003

Table 4 – 6. continued.

Parameters[a]	CA-MRSA (N=31)	HA-MRSA (N=50)	No_C (N=1622)	P vaule
Intubation	22 (71.0%)	43 (86.0%)	731 (45.1%)	<0.0001
Usage of anti_2	15 (48.4%)	16 (32.0%)	240 (14.8%)	<0.0001
Usage of anti_3	3 (9.7%)	7 (14.0%)	224 (13.8%)	0.8020
Usage of anti_4	3 (9.7%)	2 (4.0%)	329 (20.3%)	0.0063
Usage of anti_5	2 (6.5%)	1 (2.0%)	16 (1.0%)	0.0136
Usage of anti_6	6 (19.4%)	12 (24.0%)	140 (8.6%)	0.0002
Usage of anti_7	5 (16.1%)	13 (26.0%)	111 (6.8%)	<0.0001
Usage of anti_8	2 (6.5%)	9 (18.0%)	70 (4.3%)	<0.0001
Usage of anti_9	1 (3.2%)	12 (24.0%)	104 (6.4%)	<0.0001
Usage of anti_11	4 (12.9%)	7 (14.0%)	153 (9.43%)	0.4604
Usage of anti_12	2 (6.5%)	8 (16.0%)	106 (6.5%)	0.0325
Usage of anti_13	1 (3.2%)	5 (10.0%)	41 (2.5%)	0.0064
Usage of anti_14	4 (12.9%)	7 (14.0%)	52 (3.2%)	<0.0001
Usage of anti_15	1 (3.2%)	10 (20.0%)	108 (6.7%)	0.0009
Usage of aspirin	10 (32.3%)	13 (26.0%)	804 (49.6%)	0.0008
Usage of anti_17	0	4 (8.0%)	25 (1.5%)	0.0018

Abbreviation: No_C, no colonization of MRSA; dz, disease; GI, gastrointestinal; CVC, central venous catheter; A-line, arterial line; NG, nasogastric; Ca., catheter; anti_1, penicillins without anti-pseudomonal effect and not combined with β-lactamase inhibitors; anti_2, anti-pseudomonal penicillins; anti_3, penicillins combined with β-lactamase inhibitors; anti_4, first-generation cephalosporins; anti_5, second-generation

cephalosporins; anti_6, third-generation cephalosporins without anti-pseudomonal effect; anti_7, third-generation cephalosporins with anti-pseudomonal effect; anti_8, fourth-generation cephalosporins; anti_9, carbapenems; anti_10, monobactam; anti_11, glycopeptides; anti_12, anti-anaerobic agents and antibiotics with effect against atypical pathogens; anti_13, aminoglycosides; anti_14, anti-fungals; anti_15, fluoroquinolones; anti_16, colistin; anti_17, tigecycline.

[a]Parameters of autoimmune diseases, malignancy, steroid usage, usage of anti_1, usage of anti_10, and usage of anti_16 were not shown in this table because of sparse data.

Table 4 – 7. Univariate analysis of risk factors for acquiring CA-MRSA during ICU stay

[a]Parameter	CA-MRSA vs No_C		CA- v.s. HA-MRSA		P value of overall model
	OR	P value	OR	P value	
Age	1.00	0.5815	1.00	0.8597	0.8422
Gender	0.78	0.5071	0.82	0.6676	0.7960
Days at risk	1.03	0.0974	0.99	0.5727	0.0014
APACHE II > 17	3.35	0.0014	0.66	0.3959	<.0001
Smoking	0.59	0.1955	0.44	0.1029	0.2639
CV dz	1.64	0.2807	3.55	0.0181	0.0145
Respiratory dz	3.19	0.0022	0.45	0.1263	<.0001
Hepatobiliary dz	1.20	0.8029	0.22	0.0580	<.0001
Genitourinary dz	1.95	0.0671	1.34	0.5242	0.0895
GI dz	1.04	0.9379	0.77	0.6634	0.7034
Muco. dz	2.14	0.3063	3.38	0.3288	0.5298
Neurovascular dz	2.04	0.0867	1.59	0.4038	0.1896
Endocrinologic dz	2.11	0.0399	1.82	0.1945	0.1089
Recent operation	0.56	0.1257	2.20	0.1262	0.0002
CVC	2.00	0.0660	0.57	0.2677	0.0002
NG	5.23	0.0021	0.59	0.4760	<.0001
Foley	1.45	0.3683	0.25	0.0367	0.0025
ET_tube	2.72	0.0123	0.40	0.1048	<.0001
Anti_2	4.60	<.0001	1.99	0.1427	<.0001
Anti_3	0.66	0.4914	0.66	0.5674	0.7892

Table 4 – 7. Continued.

[a]Parameter	CA-MRSA v.s. No_C		CA- v.s. HA-MRSA		P-value of overall model
	OR	P-value	OR	P-value	
Anti_4	0.46	0.2050	2.57	0.3167	0.0275
Anti_5	6.56	0.0142	3.38	0.3288	0.0441
Anti_6	2.53	0.0444	0.76	0.6256	0.0004
Anti_7	2.30	0.0937	0.55	0.3030	<.0001
Anti_8	1.47	0.6051	0.31	0.1572	0.0003
Anti_9	0.44	0.4189	0.11	0.0354	0.0001
Anti_11	1.27	0.6615	0.91	0.8886	0.6663
Anti_12	0.93	0.9215	0.36	0.2191	0.0583
Anti_13	1.18	0.8698	0.30	0.2826	0.0207
Anti_14	3.83	0.0146	0.91	0.8886	0.0003
Anti_15	0.43	0.4050	0.13	0.0612	0.0041
Aspirin	0.52	0.0926	1.36	0.5444	0.0036

Abbreviation: No_C, no MRSA colonization; OR, odds ration; CV, cardiovascular; dz, diseases; GI, gastrointestinal; Muco., mucocutaneous; CVC, central venous catheter; NG, nasogastric; ET, endotracheal; anti_1, penicillins without anti-pseudomonal effect and not combined with β-lactamase inhibitors; anti_2, anti-pseudomonal penicillins; anti_3, penicillins combined with β-lactamase inhibitors; anti_4, first-generation cephalosporins; anti_5, second-generation cephalosporins; anti_6, third-generation cephalosporins without anti-pseudomonal effect; anti_7, third-generation cephalosporins with anti-pseudomonal effect; anti_8,

fourth-generation cephalosporins; anti_9, carbapenems; anti_10, monobactam; anti_11, glycopeptides; anti_12, anti-anaerobic agents and antibiotics with effect against atypical pathogens; anti_13, aminoglycosides; anti_14, anti-fungals; anti_15, fluoroquinolones; anti_16, colistin; anti_17, tigecycline.

[a]Parameters of autoimmune disease, malignancy, immune status, presence of A-line, anti_1, anti_10, anti_16, and anti_17 were not listed in the table because of sparse data leading to divergence of statistic estimate.

Table 4 – 8. Multivariate analysis of risk factors for acquiring CA-MRSA during ICU stay

Parameter	CA-MRSA v.s. No MRSA		CA-MRSA v.s. HA-MRSA		P value of
	Odds ratio (95% C.I.)	P-value	Odds ratio (95% C.I.)	P-value	overall model
NG tube	3.53 (1.16 – 10.75)	0.0262	0.50 (0.11 – 2.31)	0.3769	0.0001
Anti_2	3.09 (1.45 – 6.58)	0.0035	2.43 (0.93 – 6.38)	0.0704	0.0114
Anti_9	0.19 (0.02 – 1.45)	0.1085	0.08 (0.01 – 0.72)	0.0238	0.0240
Anti_14	3.45 (1.10 – 10.74)	0.033	1.74 (0.42 – 7.14)	0.4441	0.0411

Abbreviation: dz, diseases; NG, nasogastric; Anti_2, anti_2, anti-pseudomonal penicillins; anti_9, carbapenems; anti_14, anti-fungals.

3 Mortality of nosocomial MRSA bloodstream infection

During the three-year study period, there were 329 nosocomial MRSA bloodstream infections from 308 patients (12 patients had two episodes of MRSA bloodstream infection, 3 had three episodes, and one had four episodes). Only the first episode of each patient was enrolled for subsequent analysis. The male to female ratio was 108:200. The age distribution was 61.6±24.2 years. Among the 308 episodes, 137 episodes occurred in ICUs and 171 in general wards. One hundred and thirty-two episodes developed in patients with mechanic valve (7 patients), vascular graft (121), and orthopedic prosthesis (4). Among these 132 episodes, 80 were with prosthesis infection. In addition, 21 episodes were with infective endocarditis, and 12 episodes were with osteomyelitis. The primary foci of these 308 episodes included urinary tract (13 episodes), respiratory tract (42), surgical wound (8), skin (17), intra-abdomen (10), intravascular catheter (127), while 134 had no primary foci. The Charlson comorbidity index while onset of bloodstream infection was 7.5±6.0. One hundred and thirty-four patients developed septic shock while onset of infection. Every patient had various underlying diseases (Table 4 – 9).

The laboratory data while onset of bloodstream infection was also listed in Table 4 – 9. One hundred and ninety-five patients had abnormal white blood

cell counts, 142 had anemia, 148 had thrombocytopenia, 25 had hypoalbuminemia, 74 had abnormal liver function test, 127 had abnormal renal function, 22 had elevated LDH while onset of bloodstream infection. Two hundreds and fifty-five patients were put on effective antibiotics within 48 hours. The average time from initiation of effective antibiotics to defervesence was 4.4±6.4 days (for those who were persistently febrile till death, the time was censored on the day of death). The first antibiotics used to treat these MRSA bloodstream infection included vancomycin in 252 patients, teicoplanin in 55 episodes, and linezolid in one. The all-cause mortality rate at Day 14 was 19.8% (61 deaths). The all-cause mortality rate at Day 30 was 30.5% (94 deaths) (Table 4 – 9).

A total of 253 MRSA isolates were available for microbiologic analysis. The distribution of vancomycin MIC was 0.5 mg/L in 10 isolates, 1 mg/L in 190 isolates, and 2 mg/L in 53 isolates. One hundred and fourty-nine isolates were ST239, 47 isolates were ST59, 46 isolates were ST5, and 11 isolates belonged to other MLST types. All isolates of ST239 carried type III SCC*mec* element, 29 of the 47 isolates belonged to ST59 carried type IV SCC*mec* element, and 10 of them carried PVL gene; 18 of the 47 isolates belonged to ST59 carried type V SCC*mec* element, and all of them carried PVL gene. All 46 isolates

belonged to ST5 carried type II SCC*mec* element. Among the 11 isolates belonged to other minor MLST types, 5 carried type III SCC*mec* element, and 6 carried type II SCC*mec* element. Therefore, 47 isolates belonged to CA-MRSA strains and all these were of ST59. All the 47 CA-MRSA isolates had vancomycin MIC level less than or equal to 1 mg/L. Among the 206 HA-MRSA isolates, 153 had vancomycin MIC level less than or equal to 1mg/L, and 53 had that equal to 2 mg/L.

The patients' characteristics stratified with the causative MRSA isolates belonging to either CA-MRSA or HA-MRSA were listed in Table 4 – 10. In the group of CA-MRSA, more patients were female gender, had higher Charlson comorbidity index, had underlying hepatobiliary tract diseases, and had underlying malignancies, fewer patients developed MRSA bloodstream infection in ICU, as well as had underlying cardiovascular diseases. No difference in the distribution of mortality was noted.

The results of univariate analysis for risk factors for Day 14 mortality were listed in Table 4 – 11. Age, septic shock, duration of fever, having underlying hepatobiliary disease, thrombocytopenia, abnormal renal function, not receiving effective antibiotics with 48 hours, and MRSA isolates with vancomycin MIC equal to 2 mg/L were risk factors for all-cause Day 14

mortality.

The results of univariate analysis for risk factors for Day 30 mortality were listed in Table 4 – 12. Age, septic shock, having underlying gastrointestinal tract diseases, having underlying malignancies, thrombocytopenia, abnormal renal function, and MRSA isolates with vancomycin MIC equal to 2 mg/L were risk factors for all-cause Day 30 mortality.

The results of multivariate analysis for risk factors for Day 14 mortality were listed in Table 4 – 13. Septic shock, thrombocytopenia, and no effective treatment within 48 hours were found to be independent risk factor for mortality (OR: 13.47, 4.22, and 4.79, respectively; 95% C.I.: 5.94 – 30.55, 1.95 – 9.09, and 1.66 – 13.82, respectively; P value: <0.0001, 0.0003, and 0.0038, respectively).

The results of multivariate analysis for risk factors for Day 30 mortality were listed in Table 4 – 14. Septic shock, having underlying malignancy, anemia, thrombocytopenia, and MRSA isolates with vancomycin MIC equal to 2 mg/L were independent risk factors for all-cause Day 30 mortality (OR: 8.11, 2.49, 2.03, 3.34, and 3.69, respectively; 95% C.I.: 4.06 – 16.19, 1.25 – 4.97, 1.03 – 4.03, 1.66 – 6.76, and 1.67 – 8.14, respectively; P value: <0.0001, 0.0098, 0.0425, 0.0007, and 0.0012, respectively). CA-MRSA infection was not

associated with a poorer outcome compared to those caused by HA-MRSA.

Table 4 – 9. Demographic, clinical, laboratory, and microbiologic data of the 308 MRSA bloodstream infection episodes

Parameter	No. (mean±sd) (%)
Age (years)	61.6±24.2
Gender (male to female)	108 (35.1%)/200 (64.9%)
Charlson comorbidity index	7.5±6.0
Primary focus of bloodstream infection	
Urinary tract	13 (3.7%)
Respiratory tract	42 (12.0%)
Surgical wound	8 (2.3%)
Skin	17 (4.8%)
Intra-abdomen	10 (2.9%)
Intravenous catheter	127 (36.2%)
No obvious focus	134 (38.2%)
Location	
Icu	137 (44.5%)
General ward	171 (55.5%)
Presence of prosthesis	
Mechanic valve	7 (2.3%)
Vascular graft	121 (38.9%)
Orthopedic prosthesis	4 (1.3%)
No prosthesis	179 (57.6%)
Infection of prosthesis	
Yes	79 (61.2%)
No	50 (38.8%)

Table 4 – 9. Continued.

Parameter	No. (mean±sd) (%)
Metastatic focus of bloodstream infection	
Infective endocarditis	21 (6.8%)
Osteomyelitis	12 (3.9%)
No known	274 (89.3%)
Septic shock while onset	
Yes	129 (41.9%)
No	179 (58.1%)
Cardiovascular diseases	
Yes	191 (62.0%)
No	117 (38.0%)
Respiratory diseases	
Yes	78 (25.3%)
No	230 (74.7%)
Neurologic diseases	
Yes	67 (21.8%)
No	241 (78.3%)
Gastrointestinal tract diseases	
Yes	57 (18.5%)
No	251 (81.5%)
Hepatobiliary tract diseases	
Yes	65 (21.1%)
No	243 (78.9%)

Table 4 – 9. Continued.

Parameter	No. (mean±sd) (%)
Genitourinary tract diseases	
Yes	115 (37.3%)
No	193 (62.7%)
Endocrinologic diseases	
Yes	119 (38.6%)
No	189 (61.4%)
Malignancies	
Yes	108 (35.1%)
No	200 (64.9%)
Autoimmune diseases	
Yes	18 (5.8%)
No	290 (94.2%)
Immunosuppression	
Yes	119 (38.6%)
No	189 (61.4%)
WBC	
Leucopenia	38 (12.4%)
Normal count	112 (36.5%)
Leukocytosis	157 (51.1%)
Anemia	
Yes	142 (46.3%)
No	165 (52.7%)

Table 4 – 9. Continued.

Parameter	No. (mean±sd) (%)
Thrombocytopenia	
Yes	148 (48.2%)
No	159 (51.8%)
Hypoalbuminemia	
Yes	25 (17.7%)
No	116 (82.3%)
Abnormal liver function test	
Yes	74 (36.5%)
No	129 (63.5%)
C-reactive protein	3.43±2.93 (mg/dL)
Abnormal renal function	
Yes	127 (43.0%)
No	168 (57.0%)
Elevated lactate dehydrogenase	
Yes	22 (73.3%)
No	8 (26.7%)
Effective treatment within 48 hours	
Yes	255 (82.8%)
No	53 (17.2%)
Initial effective treatment	
Vancomycin	252 (81.8%)
Teicoplanin	55 (17.9%)
Linezolid	1 (0.3%)

Table 4 – 9. Continued.

Parameter	No. (mean±sd) (%)
Chang of antibiotics during therapy	
Yes	52 (16.9%)
No	256 (83.1%)
Day 14 all-cause death	61 (19.8%)
Day 30 all-cause death	94 (30.5%)
Duration of fever (days)	4.1±6.4
SCC*mec*	
2	52 (20.6%)
3	154 (60.9%)
4	29 (11.5%)
5	18 (7.1%)
Carriage of PVL gene	
Yes	28 (11.1%)
No	225 (88.9%)
Sequence type by MLST	
ST239	149 (58.9%)
ST59	47 (18.6%)
ST5	46 (18.2%)
others	11 (4.3%)
MIC of vancomycin (mg/L)	
0.5	10 (3.9%)
1	190 (75.1%)
2	53 (21.0%)

Table 4 – 10. Patients' characteristics stratified by type of causative MRSA

Variables	CA-MRSA (N=47)	HA-MRSA (N=206)	P value
Age	59.8±20.8	64.3±22.3	0.2081
Gender (male to female)	21/26 (44.7%/55.3%)	141/65 (68.4%/31.6%)	0.0022
Charlson comorbidity index	4.5±3.5	3.1±2.5	0.0093
Onset in the ICU	9 (19.1%)	103 (50.0%)	0.0001
Presence of metastatic foci	1 (2.1%)	27 (13.1%)	0.0298
Septic shock	14 (29.8%)	87 (42.2%)	0.1159
Primary bloodstream infection	36 (76.6%)	156 (75.7%)	0.9002
Presence of prosthesis	17 (36.2%)	87 (42.2%)	0.4459
Prosthesis with infection	10 (21.3%)	57 (27.7%)	0.3701
Cardiovascular diseases	20 (42.6%)	141 (68.4%)	0.0009
Respiratory diseases	8 (17.0%)	57 (27.7%)	0.1316
Neurologic diseases	7 (14.9%)	49 (23.8%)	0.1851
Gastrointestinal tract diseases	11 (23.4%)	34 (16.5%)	0.2644
Hepatobiliary diseases	14 (29.8%)	37 (18.0%)	0.0682
Genitourinary tract diseases	15 (31.9%)	79 (38.3%)	0.4101
Endocrinologic diseases	18 (38.3%)	87 (42.2%)	0.6213
Malignancies	23 (48.9%)	60 (29.1%)	0.0091
Autoimmune diseases	4 (8.5%)	12 (5.8%)	0.4949
Immunosuppression	21 (44.7%)	79 (38.3%)	0.4231
Abnormal WBC count	28 (59.6%)	126 (61.2%)	0.8106
Anemia	29 (61.7%)	110 (53.4%)	0.3173

Table 4 – 10. Continued

Variables	CA-MRSA (N=47)	HA-MRSA (N=206)	P value
Thrombocytopenia	25 (53.2%)	99 (48.1%)	0.5446
Hypoalbuminemia	12 (25.5%)	88 (42.7%)	0.0793
Abnormal liver function test	24 (51.1%)	79 (38.3%)	0.9044
Abnormal renal function	29 (61.7%)	110 (53.4%)	0.3929
C-reactive protein	7.0±5.7	7.4±5.8	0.7084
Elevation of LDH	1 (2.1%)	5 (2.4%)	0.4782
Effective treatment within 48 hours	38 (80.9%)	174 (84.5%)	0.5439
Day 14 mortality	7 (14.9%)	40 (19.4%)	0.4718
Day 30 mortality	12 (25.5%)	64 (31.1%)	0.4550

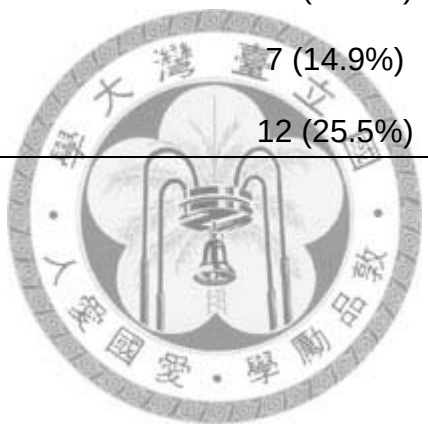


Table 4 – 11. Univariate analysis for risk factors for Day 14 mortality of nosocomial MRSA bloodstream infection

Variables	OR	95% C.I.		P value
		Lower	Upper	
Age	1.02	1.00	1.03	0.0157
Gender	1.37	0.75	2.52	0.3111
Charlson comorbidity index	1.05	0.96	1.16	0.2611
Onset in the ICU	1.38	0.78	2.41	0.2669
Presence of metastatic foci	1.10	0.45	2.66	0.8380
Septic shock	14.91	6.76	32.87	<.0001
Duration of fever	0.92	0.86	0.99	0.0347
Primary bloodstream infection	0.53	0.25	1.10	0.0868
Presence of prosthesis	0.96	0.54	1.69	0.8740
Prosthesis with infection	2.02	0.97	4.20	0.0604
Cardiovascular diseases	0.93	0.52	1.65	0.8073
Respiratory diseases	0.95	0.50	1.82	0.8829
Gastrointestinal tract diseases	1.59	0.81	3.11	0.1744
Hepatobiliary diseases	1.98	1.05	3.71	0.0339
Genitourinary tract diseases	1.44	0.81	2.53	0.2131
Endocrinologic diseases	0.67	0.37	1.21	0.1817
Malignancies	1.37	0.77	2.44	0.2804
Autoimmune diseases	0.80	0.22	2.86	0.7311
Immunosuppression	1.72	0.98	3.02	0.0605
Abnormal WBC count	1.34	0.74	2.45	0.3347
Anemia	1.55	0.87	2.75	0.1364

Table 4 – 11. Continued.

Variables	OR	95% C.I.		P value
		Lower	Upper	
Thrombocytopenia	5.71	2.84	11.49	<.0001
Hypoalbuminemia	1.92	0.61	6.02	0.2664
Abnormal liver function	1.19	0.61	2.32	0.6063
Abnormal renal function	1.92	1.08	3.41	0.0268
C-reactive protein	1.04	0.99	1.09	0.1143
Elevation of lactate dehydrogenase	3.27	0.33	31.91	0.3087
Without effective treatment within 48 hours	2.70	1.03	7.11	0.0441
SCCmec element				
Type II v.s. type IV or V	1.21	0.53	2.75	0.6515
Type III v.s. type IV or V	0.89	0.48	1.66	0.7213
Causative MRSA carrying PVL	0.71	0.15	3.29	0.6630
CA-MRSA	0.73	0.30	1.74	0.4734
MRSA vancomycin MIC=2 mg/L	2.36	1.17	4.75	0.0164

Table 4 – 12. Univariate analysis for risk factors for Day 30 mortality of nosocomial MRSA bloodstream infection

Variables	OR	95% C.I.		P value
		Lower	Upper	
Age	1.02	1.01	1.03	0.0029
Gender	1.22	0.73	2.05	0.4429
Charlson comorbidity index	1.08	1.00	1.17	0.0644
Onset in the ICU	1.56	0.96	2.54	0.0743
Presence of metastatic foci	0.98	0.45	2.16	0.9668
Septic shock	11.70	6.48	21.11	<.0001
Duration of fever	0.98	0.94	1.02	0.3652
Primary bloodstream infection	0.62	0.34	1.13	0.1181
Presence of prosthesis	0.92	0.56	1.50	0.7312
Prosthesis with infection	1.57	0.88	2.83	0.1284
Cardiovascular diseases	1.05	0.64	1.73	0.8571
Respiratory diseases	1.02	0.58	1.77	0.9557
Neurologic diseases	0.88	0.48	1.59	0.6642
Gastrointestinal tract diseases	1.72	0.95	3.11	0.0761
Hepatobiliary diseases	1.58	0.89	2.79	0.1191
Genitourinary tract diseases	0.99	0.60	1.64	0.9801
Endocrinologic diseases	0.86	0.52	1.42	0.5559
Malignancies	1.81	1.10	2.99	0.0198
Autoimmune diseases	0.64	0.20	1.98	0.4347
Immunosuppression	1.44	0.88	2.35	0.1496
Abnormal WBC count	1.25	0.75	2.08	0.3979

Table 4 – 12. continued.

Parameters	OR	95% C.I.		P value
		Lower	Upper	
Anemia	1.60	0.97	2.62	0.0642
Thrombocytopenia	4.74	2.73	8.20	<.0001
Hypoalbuminemia	2.49	0.93	6.67	0.0711
Abnormal liver function	1.01	0.56	1.84	0.9711
Abnormal renal function	2.08	1.26	3.43	0.0041
C-reactive protein	1.05	1.00	1.09	0.0505
Elevation of lactate dehydrogenase	2.50	0.41	15.23	0.3203
Without effective treatment within 48 hours	1.43	0.73	2.82	0.2997
SCC*mec* element				
Type II v.s. type IV or V	1.30	0.62	2.70	0.4847
Type III v.s. type IV or V	1.13	0.66	1.93	0.6660
Causative MRSA carrying PVL	1.30	0.42	4.01	0.6495
CA-MRSA	0.76	0.37	1.56	0.4560
MRSA vancomycin MIC=2 v.s. MIC<2 mg/L	2.89	1.54	5.40	0.0009

Table 4 – 13. Multivariate analysis for risk factors for Day 14 mortality of nosocomial MRSA bloodstream infection

Parameters	OR	95% C.I.		P vaule
		Lower	Upper	
Septic shock	13.47	5.94	30.55	<.0001
Thrombocytopenia	4.22	1.95	9.09	0.0003
No effective treatment within 48 hours	4.79	1.66	13.82	0.0038

Table 4 – 14. Multivariate analysis for risk factors for Day 30 mortality of nosocomial MRSA bloodstream infection

Parameters	OR	95% C.I.		P value
		Lower	Upper	
Septic shock	8.11	4.06	16.19	<.0001
Malignancies	2.49	1.25	4.97	0.0098
Anemia	2.03	1.03	4.03	0.0425
Thrombocytopenia	3.34	1.66	6.76	0.0007
MRSA vancomcyin MIC=2 mg/L v.s. MIC<2 mg/L	3.69	1.67	8.14	0.0012

Chapter 5 Conclusion and Discussion

Our studies demonstrated that prevalence rate of nasal carriage of CA-MRSA isolates among community adults was 3.6% (95% C.I., 2.9 – 4.3%) and its risk factors included having household members aged under 7 years , and prior antibiotic exposure within past 1 year. Smoking was found to be a significant factor inhibiting the nasal carriage of CA-MRSA. The incidence of newly acquiring CA-MRSA carriage in ICU was 3.0 per 1000 patient-days (95% C.I., 2.1 – 4.3 per 1000 patient-days) and the significant factors associated with newly acquiring CA-MRSA carriage including presence of NG tube, and prior usage of anti-pseudomoanl penicillins, carbapenems, as well as anti-fungals. The all-caused mortality rates on Day 14 and Day 30 after nosocomial MRSA bloodstream infection were 19.8% and 30.5%, respectively. The risk factors for Day 14 all-cause mortality among patients with nosocomial MRSA bloodstream infection included presence of septic shock and thrombocytopenia at presentation, as well as no effective treatment within 48 hours. The risk factors for Day 30 all-cause mortality included presence of septic shock, underlying malignancies, anemia, and thrombocytopenia at presentation, as well as causative MRSA isolate with a vancomyin MIC of 2 mg/L; but infection caused by CA-MRSA was not associated with a poorer

outcome.

As described in several previous studies, the emergence of CA-MRSA has resulted in two major impacts on clinical epidemiology, including increase of community-acquired CA-MRSA infection,[85–87] and invasion of CA-MRSA into healthcare-associated environement to cause nosocomial infection.[41–43, 104–110] Under these changes of clinical epidemiology, to understand the prevalence of and risk factors for CA-MRSA carriage among both healthy and hospitalized people, as well as the impact of CA-MRSA on the outcome of MRSA infection became more and more important. Previous studies only addressed prevalence rates of CA-MRSA among healthy people, especially the pediatric population, with limited discussion on the risk factors.[103, 106, 111–113, 128–130] Our studies were the first one to investigate the prevalence rate and risk factors for CA-MSRA nasal carriage among healthy adults in Tawan, and were the first one to disclose the incidence of and risk factors for newly acquiring CA-MRSA carriage among adult ICU patients, as well as the possible impact of CA-MRSA on the outcome of nosocomial MRSA bloodstream infection. These results were helpful for us to understand the clinical impact by CA-MRSA isolates and control its spread in both in the community and hospitals.

1. Prevalence and risk factors of nasal carriage of CA-MRSA among healthy adults

In this study, the carriage rate was 3.6% and most CA-MRSA isolates were ST59. A previous population-based study showed that the MRSA colonization rate among people attending the 2001 – 2002 NHANES was 0.84%.[100] A study in the Netherlands from 1999 to 2000 indicated that the MRSA colonization rate among the general Dutch population was 0.03%.[128] The MRSA colonization rate in this study was about 5- to 10-fold higher than reported in these prior studies. There may be several reasons for this difference. First, colonization by MRSA among adults from community may be more prevalent in Taiwan than in the U.S.A. and the Netherlands. Second, our study was conducted 5 – 7 years after those studies, so the difference may be due to an overall increase of MRSA during this time, since several previous studies have demonstrated that the MRSA colonization rate of people in the community has increased over time.[102, 106]

Multivariate analysis to identify the associated factors with nasal carriage of CA-MRSA among community adults indicated that the presence of household member less than 7 years old, and use of antibiotics within the past year were the independent risk factors for CA-MRSA nasal carriage compared

to those without *S. aureus*. Smoking was found to be a significant factor inhibiting nasal carriage of CA-MRSA compared to those without *S. aureus*. The presence of household members less than 7 years old, and use of antibiotics within the past year were the only two independent risk factors for CA-MRSA nasal carriage compared to those carrying MSSA. A previous study showed that the CA-MRSA carriage rate of Taiwanese children from the community was 7.2% in 2005 – 2006,[113] much higher than that of adults (3.6%) in the present study. The hypothesis that transmission from children to their parents through close household contact might play an important role in CA-MRSA carriage among adults is worthy of further study. We also found that use of antibiotics was associated with the presence of CA-MRSA. This was expected, because antibiotics provide selective pressure and thus facilitated the colonization of drug-resistant pathogens, such as CA-MRSA. Surprisingly, in a comparison of people with CA-MRSA and those without *S. aureus* colonization, we found that smoking was a significant factor inhibiting nasal carriage of CA-MRSA. However, a comparison of people with CA-MRSA and those with MSSA found that smoking was not such a factor. In re-analyzing our data, we found that smoking was also a significant factor inhibiting MSSA and *S. aureus* (pooling CA-MRSA and MSSA together) carriage when compared to

those without carriage of *S. aureus* (odds ratio, 0.46 and 0.46, respective; 95% confidence interval, 0.35 – 0.61 and 0.35 – 0.59, respectively; P value, <0.0001 and <0.0001, respectively). Therefore, it seems that smoking is a significant factor inhibiting nasal carriage of *S. aureus*, not only specifically against CA-MRSA. To our best knowledge, only a review article described the similar findings based on the results from a Ph.D. thesis.[130] Our study therefore provided the important evidence that smoking might inhibit nasal carriage of *S. aureus*. It might be that smoking creates a microenvironment in the nose that protects against growth of *S. aureus*.

There are limitations in our study. We only enrolled adults who attended mandatory health examinations as a part of workplace health promotion program, thus might not be representative of the adult population in the community. Since these attendees were presumably more healthy than average, our results may be biased by the healthy worker effect and possibly underestimated the carriage rate.[131] In addition, a cross-sectional study designed made us difficult to make a robust causal inference.

2. Prevalence and risk factors of carriage of CA-MRSA among ICU adult patients

The study demonstrated that 58.5% of MRSA isolated from ICU patients and 38.3% (31/81) of newly acquired MRSA during ICU stay belonged to CA-MRSA strains. The results echoed the previous findings that CA-MRSA has invaded into the hospital environment and become endemic strains.[17, 110] The prevalence rate of MRSA carriage among ICU patients was 14.9% (95% C.I., 13.3 – 16.5%) in this study, which was similar to prior reports (11.4% - 15.7%).[132–134] Previous studies seldom discussed about the incidence of newly acquirement of CA-MRSA during hospitalization. The current study demonstrated that the incidence was 3.0 per 1000 patient-days.

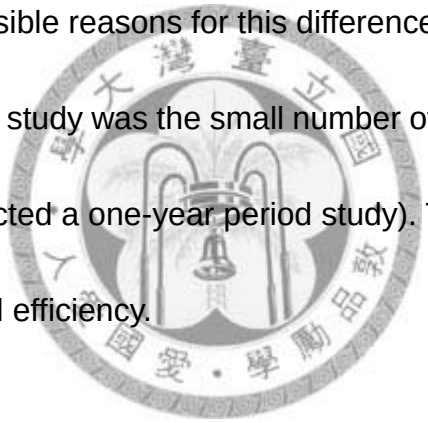
As mentioned above, risk factors to acquire CA-MRSA during ICU stay are never discussed before. Multivariate analysis in our study indicated that presence of NG tube, and prior usage of anti-pseudomonal penicillins as well as anti-fungals were independent risk factors for new acquirement of CA-MRSA carriage during ICU stay while compared to those without MRSA carriage. However, prior usage of carbapenems was a protective factor against new acquirement of CA-MRSA carriage during ICU stay while compared to those who newly acquired HA-MRSA carriage during ICU stay (Table 4 – 8). Anti-pseudomonal penicillins were broad-spectrum antibiotics without activity against MRSA.[135] All 79 patients with exposure to anti-fungals all also had

exposure to several broadspectrum antibiotics, including 28 patients to anti-pseudomonal penicillins, 41 to 3rd-generation cephalosporins, 20 to 4th-generation cephalosporins, 35 to carbapenems, 20 to fluoroquinolones, and 10 to tigecycline. Therefore, it was reasonable that prior usage of these agents would facilitate carriage of CA-MRSA by suppressing many other bacteria. However, these two variables would not be significant while we compared patients with CA-MRSA and those with HA-MRSA. Presence of NG tube provided a foreign body in the nasal cavity, thus was very likely to help the colonization of *S. aureus* over there.[136] Also, it would not be significant while we compared patients with CA-MRSA and those with HA-MRSA.

Carbapenems were extended broad-spectrum antibiotics. However, *in vitro* activity of imipenem against MRSA was tested further due to the study results. The results showed that the MIC_{90} to imipenem of HA-MRSA was 128 times of that of CA-MRSA (32 and 0.25 mg/L, respectively), and all CA-MRSA isolates were susceptible to imipenem just by *in vitro* criteria. The result was similar to the report by Takano T, et al, which also pointed out the superior *in vitro* activity of carbapenems over anti-MRSA for CA-MRSA but not for HA-MRSA.[137] Therefore, prior usage of carbapenem would be a protective factor against subsequent acquirement of CA-MRSA when compared to HA-MRSA.

In addition, MRSA isolates from CCU were more likely to be of CA-MRSA compared to those from MICU (78/188 v.s. 70/96). It was difficult to interpret this phenomenon just by our present study. However, patients admitted to MICU were more likely to have a higher APACHE II score (> 17) compared to those admitted to CCU (515/699 v.s. 174/1207, $P < 0.0001$). This meant that patients in CCU were less critically ill than those in MICU and therefore might imply that the CCU setting was more like community compared to MICU. This could be one of the possible reasons for this difference.

The limitation in this study was the small number of cases (only 31 patients, although we had conducted a one-year period study). This would compromise the statistical power and efficiency.



3. Mortality of nosocomial MRSA bloodstream infection

Our study indicated that those caused by CA-MRSA were not associated with a poorer outcome (in term of all-cause Day 14 or Day 30 mortality). And CA-MRSA contributed to 18.6% of all nosocomial MRSA bloodstream infection. Septic shock and thrombocytopenia at presentation, as well as no effective treatment within 48 hours were independent risk factors for all-cause Day 14 mortality. Septic shock, underlying malignancies, anemia and

thrombocytopenia at presentation, as well as causative MRSA isolates with a vancomycin MIC of 2 mg/L were independent risk factor for all-cause Day 30 mortality.

The previous reported mortality rate of MRSA bloodstream infection ranged from 20 to 50%.[18, 138–140] A prior study done at NTUH showed that the all-cause Day 30 mortality rate was 36.4%,[20] which was higher than the present study (30.5%). The possible cause might be that the prior study was done during 1997 to 2001, yet the present study was done during 2006 to 2008. The medical advances might result in this difference.

The reported risk factors for mortality in patients with MRSA bloodstream infection included old age, delay in effective therapy, immunosuppressive status, advanced underlying diseases, and presence of septic shock at presentation.[18–21] Our present study revealed the similar findings. However, it was interesting that delay in effective treatment was a risk factor for Day 14 mortality, not one for Day 30 mortality. Some previous studies also demonstrated that delay in effective therapy did not affect the Day 30 mortality.[20, 141, 142] It therefore was inferred that delay in effective therapy was associated with early mortality, but other factors contributed much more to later (14 to 30 days) mortality.

Presence of septic shock at presentation stood for the disease (MRSA bloodstream infection) severity when it was noted clinically. Therefore, it was reasonable that these two factors were associated with a poor outcome in term of both Day 14 and Day 30 mortality. And the similar findings has been reported repeatedly in prior studies.[18–21]

Underlying malignancies represented the unfavorable underlying conditions. Previous studies also showed that the poor underlying condition was a risk factor for mortality due to MRSA bloodstream infection.[18–21] However, in the present study, it was not a risk factor for Day 14 mortality. This might imply that underlying condition contributed more to outcome evaluated at a longer time later, but not to outcome evaluated at the more acute phase.

Anemia and thrombocytopenia were related to both disease severity and underlying condition; therefore were associated with the mortality.

Causative MRSA isolates with a vancomycin MIC of 2mg/L was found to be a significant risk factor for Day 30 all-cause mortality. Most (271/329) patients in the present study receive vancomycin as their initial treatment. Moise et al found that MRSA isolates with a vancomycin MIC equal to or over than 2mg/L were associated with prolonged bloodstream infection and poor outcome (vancomycin treatment failure).[21, 143] The present study provided

another evidence for this findings. A consensus by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of infectious Diseases Pharmacists on the therapeutic monitoring of vancomycin addressed that an infection caused by MRSA with a vancomycin MIC equal to or over than 2 mg/L (which would be considered as “susceptible” to vancomycin using criteria by CLSI) should not use vancomycin but consider alternatives, such as daptomycin and linezolid.[144] This conclusion was made based on clinical study and simulation results using pharmacokinetics/pharmacodynamics data.[144] This therefore raised the concern of selecting antibiotics to treat MRSA bloodstream infection.

Nosocomial bloodstream infection caused by CA-MRSA was not associated with a poorer outcome in our present study. However, this might be confounded. There were 53 HA-MRSA isolates with a vancomcyin MIC of 2 mg/L. However, no CA-MRSA isolates had a vancomycin MIC greater than 1 mg/L. Therefore, the impact of CA-MRSA on the outcome of MRSA bloodstream infection might be masked by their lower vanomycin MIC level, which was associated with a better outcome. A stratified analysis by vancomcyin MIC was conducted. One hundred and fifty-three episodes caused by HA-MRSA with vancomcyin MICs ≤ 1 mg/L were compared with the

47 episodes caused by CA-MRSA. Both Day 14 and Day 30 all-cause mortality were compared. However, CA-MRSA was still not a risk factor for mortality in such stratified analysis (OR: 0.94 and 1.04, respectively; 95% C.I.: 0.38 – 2.35 and 0.49 – 2.20; P value: 0.8955 and 0.9229, respectively).

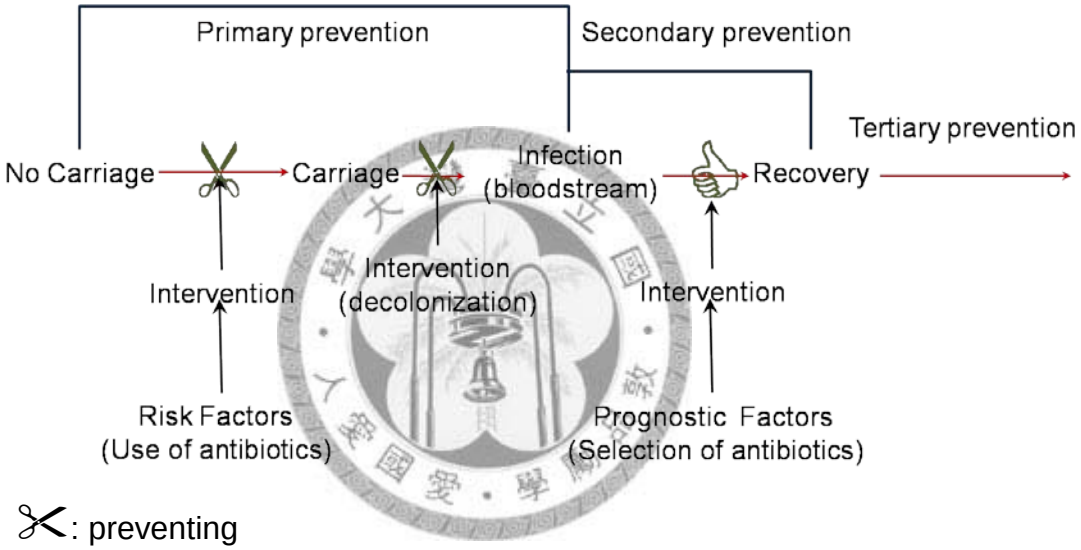
Another important finding in our study concerning nosocomial MRSA bloodstream infection was that there were 10.7% patients developed deep-seated complications (infective endocarditis, and osteomyelitis). This was also similar to prior report.[145] In addition, only 18.6% of all nosocomial MRSA bloodstream infection was caused by CA-MRSA. This is much lower than several recent reports.[42, 43] It was worthy of continuous monitoring to determine the trend of molecular epidemiology. Bloodstream infection caused by CA-MRSA was more likely to occur in general ward compared to that caused by HA-MRSA. This might be because CA-MRSA was more susceptible to several antibiotics other than β-lactams than HA-MRSA and the antibiotics selective pressure was much higher in ICU. Thus the ICU environment was against the spread of CA-MRSA.

The limitation in the study is that a retrospective study designed would lead to the uncertainty of collected variables, which in turn would compromise the statistical efficiency. In addition, small number of bloodstream infection caused

by CA-MRSA is another limitation, which might limit the statistic power in comparing the mortality of nosocomial bloodstream infection caused by CA-MRSA and HA-MRSA.

4 Implication in preventive medicine

Figure 5 – 1. Implication in preventive medicine



✂: preventing

👍: improving

For the implication in preventive medicine, our study results demonstrated prior usage of antibiotics is strongly associated with subsequent carriage of CA-MRSA isolates among adults (Figure 5 – 1). Therefore, dedicate use of antibiotics should be an important intervention to prevent or reduce subsequent CA-MRSA carriage. In our studies, we also re-emphasized that the

causative MRSA isolate with a vancomycin MIC of 2 mg/L was associated with a poorer outcome of nosocomial MRSA bloodstream infection when treated with vancomycin. Therefore, to select another effective antibiotics (such as daptomycin) in such situation, might improve the outcome (Figure 5 – 1). Using these interventions, it might help the primary and secondary preventions for MRSA infection. In addition, decolonizing MRSA using mucpirocin nasal preparation has been proven to be an effective method to prevent the subsequent MRSA infection at least among some specific population.[146] However, mupirocin nasal preparation is currently not available in Taiwan. This might make us more difficult to control / prevent MRSA infection.

Our study results had several other clinical implications. Although community-acquired *S. aureus* infection caused by CA-MRSA was not associated with a poorer outcome compared to MSSA, and the carriage rate of CA-MRSA among community adults remained low at current time, it is necessary to perform periodical surveillance on the prevalence, molecular types, and drug susceptibilities of CA-MRSA in the community settings, as well as its impact on community-acquired *S. aureus* infection. After an epidemiologic shift, it might be necessary to change the principle of empirical

antibiotics for treating community-acquired *S. aureus* infection. Because nosocomial MRSA bloodstream infection caused by CA-MRSA isolates was also not associated with a poorer outcome compared to that caused by HA-MRSA, it seemed not necessary to identify whether a nosocomial MRSA isolate was of CA-MRSA or not at current time. However, it is also necessary to continue monitoring on the epidemiology of MRSA isolates causing nosocomial infection to re-evaluate the indication of identifying whether a MRSA isolate is CA-MRSA or not periodically.

Chapter 6. Prospect

There are several further studies should be done to illuminate the questions originated from the study results addressed in this thesis. First, what is the mechanism through which smoking inhibit nasal carriage of *S. aureus*. Is it just because of the chemical effect of heat or smoke? Or is it because of some kind content in the cigarette expressing anti-*S. aureus* effect? If some chemicals with anti-*S. aureus* effect could be found, it might be helpful for controlling MRSA spread or even for treatment of infections caused by *S. aureus*. This study can be done first *in vitro* to test all possible contents in the cigarette using the method for MIC determination by CLSI.[115]

Second, the nasal carriage rate of CA-MRSA among community pediatric population is higher thean that among community adult population. The risk factors for nasal carriage of CA-MRSA among community pediatric population remain unclear in Taiwan. It therefore needs further study to disclose the possible risk factors, especially to evaluate the effect of prior usage of antibiotics on subsequent CA-MRSA carriage.

Third, based on the finding that using some β-lactams, but not all β-lactams even which had more broad-spectrum anti-bacterial effects, would predispose to subsequent acquirement of CA-MRSA, it is reasonable to

hypothesize that not all β-lactams express similar anti-CA-MRSA effect. Usually, we do not test β-lactams other than oxacillin and cefoxitin when we test drug susceptibilities for MRSA isolates.[115] Recent study also demonstrated that the same β-lactam expressed different effects against MRSA isolates belonging to different molecular types (CA-MRSA and HA-MRSA).[137] Therefore, further studies to test the susceptibilities of MSSA, CA-MRSA, HA-MRSA isolates to several β-lactams should be done. And these results might be helpful to explain the spread of CA-MRSA in the community or other environment.

Fourth, MRSA bloodstream infection caused by isolates with a vancomycin MIC equal to 2 mg/L predicted to a poorer prognosis when the infection was treated by vancomcyin; and alternatives, such as daptomycin or linezolid, were suggested to be used for treatment under this clinical condition.[143, 144] However, there is no clinical study to verify this suggestion till now. The first step to verify this suggestion is to investigate the distribution of daptomycin and linezolid MICs among the MRSA isolates with a vancomycin MIC of 2 mg/L. The second step would be to conduct a clinical study to evaluate the clinical effectiveness of dapytomycin and linezolid in treating MRSA infection caused by isolates with vancomycin MIC of 2 mg/L.

Fifth, we should conduct a prospective longitudinal study to monitor the emergence of new CA-MRSA strain. Once a new CA-MRSA strain noted, we should analyze it microbiologic and clinical features, including drug resistance, virulent factor, the mechanism facilitating its emergence, and its impact on clinical epidemiology.

Sixth, MRSA infection remains one of the infectious diseases which are difficult to treat. Therefore, to prevention is more important. Several infection control methods have been conducted to try to control the spread of MRSA, but the incidence of MRSA infection continues to increase.[147–149] This prompted many hospitals in U.S.A. to implement active surveillance and isolation (ASI) for MRSA.[149, 150] However, the results were controversial.[151–156] Whether ASI is eligible to implement and effective in Taiwan also needs further study.

Some experts consider MRSA as a perfect bug because of its ability to cause infection in nearly all parts of human body and ability to acquire new resistance to overcome the newly developed antimicrobial agents.[6] Continuous monitoring on its drug susceptibilities, molecular epidemiology, and clinical spectrum is important to improve our clinical practice and infection control against MRSA spread both in community and hospital settings.

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Appenidx 1

Questionnaire for Nasal Carriage of CA-MRSA among Health Adults

1. Age: ___ Sex: Male___ Female___ Name: _____ Study No.: ____
2. Eduation

   Less than elementary school____, elementary school____, junior high school____, senior high school____, bachelor degree____，more than master_____
3. Job: _____; working in healthcare institute: Yes____, No____
4. Marriage: married___, unmarried___, divorced___ No. of children____
5. Residential: _____ Live in a dormitory: Yes____, No____
6. No. of household members (including yourself)：___
7. Are there health care workers among your household members? Yes___, No___
8. Are there children younger than 7 years among your household members? Yes____, No____
9. Are there bed-ridden elderly among yours household members? Yes___, No___
10. Do you have any chronic disease? Yes____, No____

    If yes, please circle it: allergic rhinitis, asthma, emphysema, diabetes mellitus, hypertension, coronary artery disease, hear failure, stroke, liver cirrhosis, end-staged renal disease indicated for dialysis, cancer, autoimmune diseases, others_____
11. Smoking: Yes____, No____, Quitted____
12. Hospitalization within one year:

Yes: ____(how long ago: within 3 months, 3 – 6 months, 6 – 12 months），

reasons for hospitalization: _____

No: ____

13. Caring for inpatients within one year?

Yes: ____(how long ago: within 3 months, 3 – 6 months, 6 – 12 months），

how long you staying in hospital for caring inpatients ____ days

No: ____

14. Visiting outpatient clinic within one year

Yes: ____(how long ago: within 3 months, 3 – 6 months, 6 – 12 months），

reasons: _____, more than 12 times: Yes___, No___

No: ____

15. Using antibiotics within one year

Yes: ____(how long ago: within 3 months, 3 – 6 months, 6 – 12 months)

No: ____, Unkown: _____

16. Tattoo, acupuncture, using parenteral drug by yourself within one year

Yes: ____(how long ago: within 3 months, 3 – 6 months, 6 – 12 months),

No: ____

If yes, please circle: tattoo, acupuncture, using parenteral drug by yourself

17. Skin / soft-tissue diseases or trauma within one year

Yes: ____ (please circle: eczema, urticaria, ectopic dermatitis, folliculitis,

cellulitis, furuncle / carbuncle, acne, trauma, others _____)

No: ___

18. Shower everyday: Yes____, No____

19. Attending to the public places listed following within one year?

Yes: ___, please circle: hot spring bath, SPA, swimming pool, sauna bath,

gymnasium, dancing saloon）

No: ___

20. (Optimal) Total income of your family: (1) less than 20,000; (2) 20,000 – 50,000;(3)50,000 – 100,000; (4) 100,000 – 200,000; (5) 200,000 – 300,000; (6) more than 300,000 NTD. _____

Appendix 2

Surveillance for Carriage of CA-MRSA among ICU Adult Patients

I). Basic Information [Note: For all dates, please use mm/dd/yyyy]

Study period: ○ 1, ○ 2 Study site: ○ 3D, ○ 5C

Name:_____ Birthday: _____ Age: _____years;

Sex: ○M, ○F Date admitted to ICU: _____

Medical No.: _____ Occupation: _____

Residential: _____

Reasons for admission to ICUs:

□Septic shock; □Respiratory failure; □Heart failure; □AMI (including acute coronary syndrome); □Acute renal failure; □Post-op observation; □CABG; □ICH; □Hollow organ perforation; □Trauma; □Others,

_____

APACHE II score on first day admitted to ICUs: _____

II). Underlying diseases and risk factors:

□Cardiovascular diseases: □HCVD; □VHD; □CAD; □Cardiomyopathy;

□Heart failure; □Others_____

☐ Respiratory diseases: □COPD; □Respiratory failure;

□Others_____

□Hepatobiliary diseases: □Liver cirrhosis, de-compensated: □yes / □no;

□Hepatitis, □HBV/□HCV/□Alcohol/□Others related;

□Cholelithiasis; □Others _____

□Genitourinary disease: □Chronic renal insufficiency; □ESRD; □Acute renal

failure; □Nephrotic syndrome; □Others_____

□Gastroenterologic disease: Specified _____

□Mucocutaneous diseases: Specified _____

□Neurovascular diseases: □CVA; □Degenerative diseases;

□Others _____

□Endocrinologic diseases: □D.M.; □Others_____

□Autoimmune diseases: □SLE; □RA; □Others_____

□Malignancy: □Solid, □Lung Ca. / □HCC / □Colon Ca. / □ Gastric Ca. /

□Breast Ca. / □NPC / □Others, _____; □Hematology,

□Leukemia / □Lymphoma

□Immune suppressive medication: □steroid; □FK506; □Cellcept;

□Others_____

□Recent operation: operation site □Head; □Neck; □Chest; □Abdomen;

□GU tract; □Others _____

Smoking: □yes, ____ PPD for ____ years □no;

Alcoholism: □yes, for _____ years □no

APACHE II score at admission: _____

III). Invasive procedures while in ICUs

☐ Catheterization to sterile sites: □CVC; □A-line; □chest tube;

□surgical drainage; □CSF drainage; □Others:_____

Beginning Date: _____; End Date: _____

☐ Catheterization to mucosa: □NG; *Foley; *ET tube;

*Others: _____;

Beginning Date: _____; End Date: _____

IV). Antimicrobial and Aspirin received during this hospitalization（since 15 days before enrollment）

Antibiotic name	IV/IM/PO	Start date	Stop date

V). Hospital Microbiology (please make a copy of lab report is available):

☐ Positive for *Staphylococcus aureus*: First MRSA date:_____

Date: _____; Specimen: (blood, sputum, urine, ascites, pleural effusion, pus, others _____); OXA: (□R / □S)　(OXA, oxacillin)

Date: _____; Specimen: (blood, sputum, urine, ascites, pleural effusion, pus, others _____); OXA: (□R / □S)

Date: _____; Specimen: (blood, sputum, urine, ascites, pleural effusion, pus, others _____); OXA: (□R / □S)

VI). Final outcome while leaving ICU: ICU discharge date:_____

☐ Improved; □Stationary; □ Voluntary discharge

☐ Death not related to MRSA or ABA infections

☐ Death related to □MRSA or □ABA infection

☐ No / □ Yes for MRSA colonization: date：_____

Appendix 3

Mortality of Nosocomial MRSA Bloodstream infection

1. Demographic data

Name: Chart No.: Age: Years

Sex: □Male □Female

2. Clinical features

a. Reason for admission:

_____

b. Underlying diseases:

□No □Yes: cardiovascular disease, specified: _____

□No □Yes: respiratory disease, specified: _____

□No □Yes: neuropsychologic disease, specified: _____

□No □Yes: gastrointestinal tract disease, specified: _____

□No □Yes: hepatobiliary tract disease, specified: _____

□No □Yes: genitourinary tract disease, specified: _____

□No □Yes: endocrinologic disease, specified: _____

□No □Yes: neoplasmic disease, specified: _____

□No □Yes: autoimmune disease, specified: _____

□No □Yes: immunosuppressive agents in recent one month, specified:

_____, maximum dose _____

McCabe criteria: □rapidly fatal; □ultimately fatal; □non fatal

c. Date of onset of MRSA bloodstream infection:

_____

F/U positive blood cultures for MRSA: _____

d. Primary focus of MRSA bloodstream infection: □primary; □UTI; □RTI;

□SSI; □Skin (□catheter related □or not, in term of primary bloodstream infection)

e. Duration to deverfescence: _____ days

f. Presence of prosthesis: □Yes; □No

□mechanic vavle; □vascular graft; □orthopedic prosthesis

Infection or not: □Yes; □No Removal or not: □Yes; □No

g. Extent of MRSA bloodstream infection:

□cardiac echo prove IE; □clinically suspected IE; □deep-seated infection (osteomyelitis, visceral abscesses, etc..) by image studies

h. Severity of MRSA bloodstream infection:

□sepsis; □septicemia; □septic shock

3. Laboratory data while onset of MRSA bloodstream infection

a. Hemogram and Blood Chemistry Study while enrollment

RBC	WBC	Platelet	MCV	Hct	Hb	Meta.
Band	Seg.	Eos.	Baso.	Lymph		

Albumin	Globulin	Bil. T/D	GOT	GPT	ALP	□GT
LDH	BUN	Creatinine	CRP	Na	K	Cl

b. Hemogram and Blood Chemistry Study after during treatment:

Date	RBC	WBC	Platelet	MCV	Hct	Hb	Meta.

Date	Band	Seg.	Eos.	Baso.	Lymph		
Date	Albumin	Globulin	Bil.T/D	GOT	GPT	ALP	□GT
Date	LDH	BUN	Creatinine	CRP	Na	K	Cl

c. Drug susceptibility of MRSA

Susceptible to: _____

Resistant to: _____

d. MIC level of MRSA to vancomycin: _____μg/mL

4. Treatment:

a. Initial effective treatment within 48 hours: □No □Yes

b. Initial antibiotics: □vancomycin □teicoplanin □daptomycin □linezolid

5. Outcome: □Survive, discharged on _____; □Death, on_____

(Right censored on Mar. 31, 2009)

Appendix 4. Publications (the underlined relating to the thesis)

1. **Wang JT**, Fang CT, Chen YC, Wu CL, Chen ML, Chang SC. Staphylococcal Cassette Chromosome *mec* in Methicillin-resistant *Staphylococcus aureus*, Taiwan. Emerg Infect Dis 2007;13:494 – 497.
2. **Wang JT**, Sheng WH, Wang JL, Chen D, Chen ML, Chen YC, Chang SC. Longitudinal analysis of chlorhexidine susceptibilities of nosocomial methicillin-resistant Staphylococcus aureus isolates at a teaching hospital in Taiwan. J Antimicrob Chemother 2008;62:514 – 517.
3. **Wang JT**, Laio CH, Fang CT, Chie WC, Lai MS, Lauderdale TL, Lee WS, Huang JH, Chang SC. Prevalence and risk factors for colonization by methicillin-resistant Staphylococcus aureus among Taiwanese adults in the community. J Clin Microbiol 2009;47:2957 – 2963.
4. **Wang JT**, Lauderdale TL, Lee WS, Huang JH, Wang TH, Chang SC. Impact of active surveillance and contact isolation on transmission of methicillin-resistant Staphylococcus aureus (MRSA) in intensive care units in an area with high prevalence of MRSA. J Formos Med assoc 2010; in print.
5. Huang YT, Chang SC, Lauderdale TL, Yang AJ, **Wang JT***. Molecular epidemiology of carbapenem-resistant Pseudomonas aeruginosa carrying metallo-beta-lactamase genes in Taiwan. Diagn Microbiol Infect Dis 2007;59:211-216. (*Corresponding author)
6. Sheng WH, **Wang JT**, Lauderdale TL, Wong CM, Chen D, Chang SC. Epidemiology and Susceptibilities of Methicillin-resistant Staphylococcus aureus in Taiwan: emphasis on chlorhexidine susceptibility. Diagn Micrbobiol Infect Dis 2009; 63:309–313. (The first and second authors are equally contributory to this study)

7. Tseng YC, **Wang JT**, Wu FLL, Chen YC, Chie WC, Chang SC. Prognosis of adult patients with bloodstream infection caused by extensively-resistant Acinetobacter baumannii. Diagn Microbiol Infect Dis 2007;59:181–190. (The first and second authors are equally contributory to this study)
8. Tsai HT, **Wang JT**, Chen CJ, Chang SC. Association between antibiotic usage and subsequent colonization or infection of extensive drug-resistant Acinetobacter baumannii: a matched case-control study in intensive care units. Diagn Microbiol Infect Dis 2008; 62:298–305. (The first and second authors are equally contributory to this study)
9. Fang CT, Shau WY, Hsueh PR, Chen YC, **Wang JT**, Hung CC, Huang LY, Chang SC. Early empirical glycopeptide therapy for patients with methicillin-resistant Staphylococcus aureus bacteraemia: impact on the outcome. J Antimicrob Chemother 2006;57:511–519.
10. Sheng WH, **Wang JT**, Lin MS, Chang SC. Risk factors affecting in-hospital mortality in patients with nosocomial infections. J Formos Med Assoc 2007;106:110–118.
11. Wang JL, **Wang JT**, Chen SY, Hsueh PR, Kung HC, Chen YC, Chang SC. Adult methicillin-resistant Staphylococcus aureus bloodstream infection in Taiwan: clinical significance of non-multiresistant antibiogram and Panton-Valentine Leukocidin gene. Diagn Microbiol Infect Dis 2007: 59:365–371.
12. Wang JL, Chen SY, **Wang JT**, Wu GH, Chiang WC, Hsueh PR, Chen YC, Chang SC. Comparison of both clinical features and mortality risk associated with bloodstream infection due to community-acquired methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus. Clin Infect Dis 2008;46:799–806.

Abbreviations (listed in alphabetic order):

A-line	Arterial line
Anti_1	penicillins without anti-pseudomonal effect and not combined with β-lactamase inhibitors
Anti_2	Anti-pseudomonal penicillins
Anti_3	Penicillins combined with β-lactamase inhibitors
Anti_4	First-generation cephalosporins
Anti_5	Second-generation cephalosporins
Anti_6	Third-generation cephalosporins without anti-pseudomonal effect
Anti_7	Third-generation cephalosporins with anti-pseudomonal effect
Anti_8	Fourth-generation cephalosporins
Anti_9,	Carbapenems
Anti_10	Monobactam
Anti_11	Glycopeptides
Anti_12	anti-anaerobic agents and antibiotics with effect against atypical pathogens
Anti_13	Aminoglycosides

Anti_14	Anti-fungals
Anti_15	Fluoroquinolones
Anti_16,	Colistin
anti_17	Tigecycline
APACHE II	Acute Physiology and Chronic Health Evaluation II
ATCC	American Type Culture Collection
Ca	Catheter
CA-MRSA	Community-associated methicillin-resistant *S. aureus*
CCU	Coronary care unit
C.I.	Confidencie interval
CLSI	The Clinical and Laboratory Standards Institute
CRP	C-reactive protein
CV	Cardiovascular
CVC	Central venous catheter
dz	Diseases
ET	Endotracheal
FEMH	Far Eastern Memorial Hospital
GI	Gastrointestinal
HCW	Healthcare worker

HA-MRSA	Healthcare-associated methicillin-resistant *S. aureus*
ICU	Intensive care unit
KTC	Taipei Cathay General Hospital
LDH	Lactate dehydrogenase
MIC	Minimum inhibitory concentration
MICU	Medical intensive care unit
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *S. aureus*
Muco	Mucocutaneous
NCCLS	National Committee for Clinical and Laboratory Standards
NHANES	National Healthy and Nutrition Examination Survey
NG	Nasogastric
No_C	No colonization
NTD	New Taiwan dollar
NTUH	National Taiwan University Hospital
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PVL	Panton-Valentine leukocidine

PFGE	Pulsed-field gel electrophoresis
SAS	Statistics Analysis System
SBA	Sheep blood agar
SD	Standard deviation
SCC*mec*	Staphylococcal cassette chromosome *mec*
ST	Sequence type
WFH	Wanfang Hospital