Review article

Community- and livestock-associated methicillin-resistant *Staphylococcus aureus*: a silent threat to Thai public health

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Abstract

Methicillin-resistant *Staphylococcus aureus* or MRSA was first recognized 50 years ago as the "Superbug" in the United Kingdom as its antibiotic treatment often was limited to only vancomycin. Since then the spread of MRSA among patients in hospitals has been reported worldwide. The organism is known as hospital-associated MRSA (HA-MRSA) and currently considered one of the leading causes of morbidity and mortality of patient. During the past 15 years, significant increase of MRSA infections outside the healthcare settings has posted global public health concern. These infections are caused by MRSA strains that are genetically distinct from HA-MRSA strains so called community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). Comparatively, CA-MRSA strains are more susceptible to antimicrobial agents, rapidly spreading and often capable of causing more severe diseases such as necrotizing pneumonia and sepsis. Recent survey and molecular characterization of MRSA from Thai veterinarians revealed a LA-MRSA strain that carry a novel SCC*mec* type IX, which is to date only found in Thailand. It was classified as sequence type ST398 and *spa* type t034. In addition, the second most prevalent clonal complex (CC) among LA-MRSA strains, CC9, was isolated from pigs and pork samples in Thailand. All isolates belong to *spa* type t337 and also harbor SCC*mec* type IX. These reports highlight the possible transmission of a unique MRSA clone from livestock to human in the community, and eventually in the hospital, otherwise in the other way round. The purpose of this review was to describe the significance, molecular evolution and typing methods of MRSA and review molecular pathogenesis and epidemiology of CA-MRSA and the recent emergence of LA-MRSA in Thailand, so as to incite vital surveillance and intervention, which are necessary for control and prevention of spread of these neglected pathogens. ***Bull Chiang Mai Assoc Med Sci 2013; 46(3): 187-206***

Keywords: *Methicillin-resistant Staphylococcus aureus (MRSA), community-associated MRSA (CA-MRSA), livestock-associated MRSA (LA-MRSA)*

Methicillin-resistant *Staphylococcus aureus* ที่สัมพันธ์กับชุมชนและปศุสัตว์: ภัยเงียบของการสาธารณสุขไทย

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แขนงวิชาจุลชีววิทยาคลินิก ภาควิชาเทคนิคการแพทย์ คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่

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บทคัดย่อ

เชื้อ Methicillin-resistant *Staphylococcus aureus* หรือ MRSA เป็นที่รู้จักครั้งแรกเมื่อ 50 ปีก่อน ที่ประเทศอังกฤษ ในนาม “Superbug” เนื่องจากการรักษาเชื้อด้วยยาปฏิชีวนะมักจำกัดอยู่กับยา vancomycin เพียงชนิดเดียว ตั้งแต่นั้นเป็นต้นมามีรายงานการแพร่กระจายของ MRSA ในผู้ป่วยที่พักรักษาตัวในโรงพยาบาลทั่วโลก เชื้อนี้เป็นที่รู้จักในชื่อ hospital-associated MRSA (HA-MRSA) และปัจจุบันเป็นที่ยอมรับว่าเป็นสาเหตุอันดับต้น ๆ ของการก่อโรคและการเสียชีวิตของผู้ป่วย ในช่วงเวลา 15 ปีที่ผ่านมา การเพิ่มสูงขึ้นของการติดเชื้อ MRSA ภายนอกโรงพยาบาล ส่งผลให้เกิดความวิตกกังวลต่อปัญหาสาธารณสุขทั่วโลก การติดเชื้อเหล่านี้เกิดจากเชื้อ MRSA สายพันธุ์ที่มีลักษณะทางพันธุกรรมแตกต่างไปจากสายพันธุ์ของเชื้อ HA-MRSA เรียกว่า community-associated MRSA (CA-MRSA) และ livestock-associated MRSA (LA-MRSA) เมื่อนำมาเปรียบเทียบกัน สายพันธุ์ของเชื้อ CA-MRSA มักมีความไวต่อสารต้านจุลชีพมากกว่า มีการแพร่กระจายที่รวดเร็วและมักมีความสามารถในการก่อโรคที่รุนแรงกว่า การสำรวจและการศึกษาลักษณะทางอณูวิทยาของ MRSA ที่แยกได้จากสัตวแพทย์ชาวไทยเมื่อไม่นานมานี้ ทำให้พบเชื้อ LA-MRSA สายพันธุ์ที่มี SCC*mec* type IX ที่เพิ่งค้นพบใหม่ ซึ่งในปัจจุบันพบในประเทศไทยเท่านั้น เชื้อนี้จำแนกได้เป็น sequence type ST398 และ *spa* type t034 นอกจากนี้ ยังพบ clonal complex (CC) ที่พบบ่อยเป็นอับดับสองในบรรดาสายพันธุ์ของเชื้อ LA-MRSA คือ CC9 จากหมูและตัวอย่างเนื้อหมูในประเทศไทย ทุก isolates มี *spa* type t337 และมี SCC*mec* type IX เช่นกัน รายงานเหล่านี้ย้ำให้เห็นถึงความเป็นไปได้ในการแพร่เชื้อ MRSA สายพันธุ์ที่มีลักษณะเฉพาะนี้จากปศุสัตว์สู่คนในชุมชนและในโรงพยาบาลในที่สุด หรืออาจเกิดการแพร่เชื้อในทิศทางตรงกันข้าม นิพนธ์ปริทรรศน์นี้มีวัตถุประสงค์เพื่ออธิบายความสำคัญ วิวัฒนาการและวิธีการจำแนกชนิดทางโมเลกุลของเชื้อ MRSA และวิจารณ์พยาธิกำเนิดและระบาดวิทยาทางโมเลกุลของเชื้อ CA-MRSA ตลอดจนอุบัติการของเชื้อ LA-MRSA ที่เกิดขึ้นเมื่อไม่นานมานี้ในประเทศไทย ทั้งนี้ เพื่อกระตุ้นให้เห็นถึงความสำคัญเร่งด่วนในการเฝ้าระวังและการใช้มาตรการแทรกแซงที่จำเป็นสำหรับการควบคุมและป้องกันการแพร่ระบาดของเชื้อที่ไม่ได้รับความสนใจเหล่านี้ ***วารสารเทคนิคการแพทย์เชียงใหม่ 2556; 46(3): 187-206***

คำหัส: *Methicillin-resistant Staphylococcus aureus (MRSA), community-associated MRSA (CA-MRSA), livestock-associated MRSA (LA-MRSA)*

Introduction

Staphylococcus aureus, a non-motile gram-positive bacterium, has been recognized as a successful commensal organism and an important human pathogen. This organism can cause various human illnesses ranging from a minor infection of skin to life-threatening infections such as infectious endocarditis and sepsis. Soon after the use of methicillin, the semi-synthetic penicillin, to combat the rise of beta-lactamase producing *S. aureus* in 1960, the first case of methicillin-resistant *S. aureus* (MRSA) infection was reported in the United Kingdom in 1961. Resistance to methicillin of MRSA is due to the production of penicillin-binding protein 2a or 2' (PBP2a or PBP2') that encoded by *mecA* gene. This gene is located on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). Unlike the native PBPs, PBP2a has low affinity to beta-lactams. Therefore, cross-linkage by transpeptidase that essential for cell wall synthesis of *S. aureus* is inhibited.[1] This results in ineffective treatment of MRSA by all beta-lactam antibiotics including penicillins, penicillinase-stable penicillins (e.g. methicillin, nafcilin, oxacillin, etc.) and cephalosporins.

MRSA strains often are resistant to most common antibiotics and this makes them more difficult to treat. Higher mortality rate in patients with blood stream infection associated with MRSA compared with methicillin-susceptible S. *aureus* (MSSA) has been demonstrated.[2] In 2005, an estimated 94,000 MRSA infections resulted in more than 18,000 deaths in the United States while the lesser deaths, around 16,000 were caused by HIV/AIDS in the same period.[3, 4] Human MRSA infection is not only one of the leading causes of death but also the burden of serious economic costs of patients, hospitals and society because patients isolation, longer hospitalization and more extensive treatments are required.[5, 6] The study data in 2007 from the European Center for Disease Prevention and Control showed that 171,200 MRSA infections occurred in European Union member states plus Iceland and Norway, resulting in 1,050,000 extra days spent in the hospital. This translates into an enormous economic burden.[7]

Currently, four categories of MRSA have been categorized based on their putative sources of human infections: (i) hospital–associated MRSA (HA-MRSA), (ii) community-associated MRSA (CA-MRSA), (iii) HA-MRSA with community onset (HACO-MRSA), and (iv) livestock-associated MRSA (LA-MRSA). HA-MRSA is well known for its spread in healthcare settings worldwide. Persistence of MRSA in patient and staff skin and mucous membrane, and healthcare environments provide continual spread of MRSA in hospitals.[8] HA-MRSA prevalent rates are varied widely according to the geographical differences and the implementation of infection control measure. CA-MRSA was first reported in 1993, in Aboriginal patients with skin and soft tissue infections who live in remote communities of Western Australia.[9] CA-MRSA cases in human have been recognized since the mid 1990s in Japan.[10] However, the dramatic rise of CA-MRSA implicated in nosocomial infections have been noted from the early-2000s and until now emergence of CA-MRSA strains with highly diverse genetic backgrounds has been reported in many countries around the world.[11, 12] In the United States, CA-MRSA infections (previously restricted among injection drug users) currently are endemic in most regions both in hospitals and community. This public health crisis has alarmed the rest of world for active surveillance and control of CA-MRSA spread.[13, 14]

Unlike HA-MRSA, CA-MRSA infections often occur among young children and healthy adults with no risk factors of hospital-associated infection (HAI).[15] In 2000, the Center for Disease Control and Prevention (CDC) created the guideline to distinguish CA-MRSA from HA-MRSA based on patient medical history: the persons categorized as having CA-MRSA infection if they are diagnosed for an outpatient or within 48 h after admission to the hospital, have no history of infection or colonization by MRSA, and no permanent indwelling catheters cutaneous device, and lack the following HA-MRSA risk factors in the past year: hemodialysis, surgery, residence in a long-term care facility or hospitalization.[16] However, the epidemiology of CA-MRSA in hospital settings has become increasingly complicated whereas HA-MRSA strains occur to circulate in the community. Therefore, the third category of MRSA infections,

HA-MRSA with community onset (HACO-MRSA), has been initiated by the CDC investigators. HACO-MRSA includes HA-MRSA infections by history of health care exposure but have onset in the community.[3, 17] Despite their clinical epidemiology, distinct genotypes and phenotypes of CA-MRSA strains have been recognized. The CA-MRSA clones can be distinguished from those of HA-MRSA using *SmaI* macrorestriction of *S. aureus* genomic DNA and pulsed field gel lectrophoresis (PFGE) analysis. Molecular typing techniques such as multilocus sequence typing (MLST) and staphylococcal protein A (*spa*) and SCC*mec* typing also have been widely used to characterize MRSA lineages.[18,19] Compared with HA-MRSA, CA-MRSA grow faster and rarely resistant to non-bata-lactam antibiotics.[13, 14, 20, 21]

The last category, livestock-associated MRSA (LA-MRSA) has recently been included to describe human MRSA infection associated with exposure to livestock.[22] Although, the MRSA in animals was first implicated in bovine mastitis since 1972,[23] LA-MRSA was recognized later when it increasingly colonized and infected human in the early 2000s.[24, 25] Colonization of MRSA in animals has been extensively investigated, especially in European countries. Until now, a wide variety of animal species, mainly livestock including pigs, castles, poultry, horses, and dogs, as well as foods of animal origins have been reported as reservoirs of LA-MRSA worldwide.[26]

The role of Panton-Valentine leukocidin (PVL) in pathogenesis of CA-MRSA

The majority of CA-MRSA infections are skin and soft tissue infection, however, the CA-MRSA cases have frequently associated with rapidly progressive, life-threatening diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis. In addition, CA-MRSA infections have sometimes associated with other severe syndromes such as purpura fulminans with toxic shock syndrome,[27] and Waterhouse-Friderichsen syndrome.[28] There are various virulence factors described for pathogenesis of HA-MRSA and CA-MRSA, both surface components (e.g. capsular polysaccharide and protein A) and secreted protein (e.g. coagulase, hemolysins, enterotoxins and toxic-shock syndrome toxin).[29] Unlike HA-MRSA, extensive evidences strongly support the association of Panton-Valentine leukocidin (PVL) exotoxin with enhanced virulence of CA-MRSA.[15, 29, 30] Nevertheless, a report demonstrated that alpha-hemolysin and not PVL was responsible for death in a mouse pneumonia model, using USA300 and USA400 CA-MRSA strains.[31] PVL is the two-component protein consisted of lukS-PV and lukF-PV subunits encoded by the lukS-PV and lukF-PV genes *(pvl)*.[32] The model for emergence of the PVL-positive CA-MRSA strains demonstrated that *pvl* is likely to be acquired from PVL-positive MSSA strains by transduction of phage (phiSLT) prior to the acquisition of SCC*mec* on the different integration sites of *S. aureus* genome.[33]

PVL is capable of polymorphonuclear leukocytes (PMN) lysis by pore-formation of leukocyte membrane.[34] The death of PVL-positive CA-MRSA infected patients presenting with severe sepsis and necrotizing pneumonia has been reported to occur within 24–48 h of hospitalization.[35] Although the role of PVL in dermonecrosis in rabbits has been established,[36] the evidence for its role in severe diseases initiated by CA-MRSA infection has been limited. Recent review proposed possible steps leading to necrotizing pneumonia and severe sepsis that might mediated by PVL concentration-dependent PMN cytolysis and apoptosis.[37] The PVL subunits are secreted from MRSA cells. Then lukS-PV initiates binding to an unidentified receptor on PMN membranes and dimerized with lukF-PV. After an alternate serial binding of LukF-PV and LukS-PV components, a pore-forming heptamer is assembled and resulting in cell lysis. This may cause cells such as neutrophils to release inflammatory enzymes and cytokines. At low level of PVL, apoptosis of PMN occurs via a novel pathway that presumably involves PVL-mediated pore formation in the mitochondrial membrane. This leads to release of cytochrome *c* and induction of caspases 9 and 3 and eventually DNA fragmentation. However, at higher level, PVL will induce cytolysis of PMN. It was suggested that tissue necrosis might result from release of reactive oxygen species (ROS) or inflammatory mediators from lysed PMNs rather than direct damage by PVL.[37]

Molecular evolution of MRSA

Resistance to methicillin and all other beta-lactam antibiotics of *S. aureus* has been described that it is the result of *mecA* gene, a 2.1-kb gene that codes for the 78-kDa penicillin binding protein 2a (PBP2a) or PBP2'. The *mecA* gene is regulated by the repressor *mecI* and the transmembrane beta-lactam sensing signal transducer, *mecR1*. In the absence of beta-lactam antibiotics, *mecI*, bound to the *mecA* operator region, represses the transcription of *mecA* and *mecR1-mecI*. Conversely, in the presence of beta-lactam antibiotics, *mecR1* is auto-catalytically cleaved resulting in an active metalloprotease (located in the cytoplasmic part of *mecR1*) that enabling cleavage of the repressor *mecI*. This allows the transcription of *mecA*, and subsequent production of PBP2a to occur.[38] The *mec* complex, which is comprised of *mecA* and its divergently transcribed regulatory genes, is located on a mobile genetic element named "the staphylococcal cassette chromosome *mec* (SCC*mec*)".[39]

The mobility of SCC*mec* has been known to contribute to the cassette chromosome recombinase (*ccr*) genes of the invertase/resolvase family located on this element. The encoded recombinase proteins permit integration into and excision of the SCC*mec* from the *S. aureus* chromosome at a specific site called "SCC*mec* attachment site (attBscc)". The location of the attBscc is closed to the 3' end of *orfX* (an open reading frame with unknown function located near the replication origin, *oriC,* on the chromosome).[40] These genetic exchanges permit phenotypic alteration between methicillin-resistant and methicillin-susceptible staphylococcal species. In addition, it was found that only selected clones of *S. aureus* were able to maintain, transcribe, and translate a plasmid-born *mecA* gene.[41] This might contribute to limited number of MRSA clones that are currently disseminating worldwide: CC1, CC5, CC8, CC22, CC30, and CC45.[38] The regions outside the *mec* complex and the *ccr* genes are named "J" (Junkyard) regions, carrying a wide variety of mobile genetic elements i.e. insertion sequence (IS), transposon and plasmid. The J1 region is the region between the *ccr* genes and the chromosome right junction and the J2 region is located between the *ccr* genes and the *mec* complex. The J3 region includes the distance from *mec* complex to the left extremity (orfX) of SCC*mec*.[42, 43] Therefore, a SCC*mec* element typically has the structure as followed: J3-*mec*-J2-*ccr*-J1 (Figure 1).

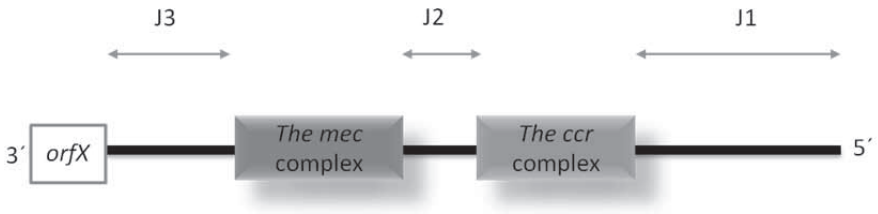

Figure 1 The structure of SCC*mec* element typically found in MRSA strains. orfX is an open reading frame of unknown function located near the origin for replication (*oriC*) at the 3'end. The mec complex includes genes that are responsible for methicillin resistance whereas the *ccr* complex encodes for recombinase protein rendering mobility of SCC*mec* element. J1, J2 and J3 stand for the junkyard regions 1, 2 and 3, respectively.

At presence, five classes of the *mec* complexes, including class A, B, C1, C2 and E, and eight types of the *ccr* genes (A1B1, A2B2, A3B3, A4B4, C, A1B6 and A1B3) have been described in *S. aureus*.[44] Given that, the SCC*mec* elements are now classified into 11 types (SCC*mec* type I to XI) based on the combination of these two components that are essential for beta-lactam resistance and mobility of SCC*mec* (Table 1). Additionally, the subtypes of SCC*mec* can be identified according to the differences of the J regions.[44] For instance, SCC*mec* type IV that related to CA-MRSA strains has a high diversity of J regions and so far contains at least 9 identified subtypes: a to h designated as SCC*mec* types IVa - IVh. This may indicate the high frequency of horizontal gene

transfer (HGT) of this SCC*mec* element. Previous studies have demonstrated that SCC*mec* types I-III are associated with HA-MRSA lineages, but SCC*mec* types IV and V are typically related to CA-MRSA. The types I, II and III SCC*mec* usually include J regions inserted with genes conferring non-beta-lactam resistance to *S. aureus*. Therefore, these strains are more commonly associated with MDR phenotypes,[13] It was found that the acquisition rate of SCC*mec* types IV to generate MRSA clones was two-times higher than that of other SCC*mec* types.[45] Relatively small sizes of SCC*mec* types associated with community infection might facilitate the mobility and exchanges of these SCC*mec* among staphylococci. This may contribute to highly diverse genetic traits and capability to spread widely as a successful human pathogen both in community and hospitals of CA-MRSA clones.[13, 38]

Table 1 Presently known SCC*mec* types in *Staphylococcus aureus* strains.

SCC*mec* types	The *ccr* complex	The *mec* complex	Origin	Year of isolation
I	Type-1 (*ccrA1B1*)	class B (IS*1272*-Δ*mecR1-mecA*-IS*431*)	UK[40]	1961
II	Type-2 (*ccrA2B2*)	class A (*mecI-mecR1-mecA*-IS*431*)	Japan[60]	1982
III	Type-3 (*ccrA3B3*)	class A (*mecI-mecR1-mecA*-IS*431*)	New Zealand[60]	1985
IV	Type-2 (*ccrA2B2*)	class B (IS*1272*-Δ*mecR1-mecA*-IS*431*)	USA[89]	1999
V	Type-5 (*ccrC*)	class C2 (IS*431*-Δ*mecR1-mecA*-IS*431*)	Australia[90]	1999
VI	Type-4 (*ccrA4B4*)	class B (IS*1272*-Δ*mecR1-mecA*-IS*431*)	Portugal[58]	1996
VII	Type-5 (*ccrC*)	class C1 (IS*431*-Δ*mecR1-mecA*-IS*431*)	Sweden[91]	2002
VIII	Type-4 (*ccrA4B4*)	class A (*mecI-mecR1-mecA*-IS*431*)	Canada[92]	2003
IX	Type-1 (*ccrA1B1*)	class C2 (IS*431*-Δ*mecR1-mecA*-IS*431*)	Thailand[46]	2006
X	Type-7 (*ccrA1B6*)	class C1 (IS*431*-Δ*mecR1-mecA*-IS*431*)	Canada[46]	2006
XI	Type-8 (*ccrA1B3*)	class E[1] (*blaZ-mecA*$_{LGA251}$*-mecR1-mecI*)	UK[47]	2007

[1] *blaZ*, a plasmid-born penicillin-resistance gene; *mecA*$_{LGA251}$, a homologue of *mecA* gene

Like CA-MRSA, the SCC*mec* types IV and V has usually been described in ST398 MRSA, the most prevalent linages that widely distributed in animals and causing livestock-associated infection in human.[26] These types of SCC*mec* often contain genes for co-resistance to non-bata-lactam antibiotics including erythromycin, trimethoprim, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, as well as those commonly used in animal production.[26] However, recent studies reported novel SCC*mec* types associated with livestock including SCC*mec* type IX, X, and XI.[46, 47] These types have been found to carry many MGEs carrying drug resistance and heavy metals resistance determinants inserted into the J regions.[46, 47] These unique traits of LA-MRSA may have evolved for some time and subsequently horizontally acquired several genetic determinants required for their survival, presumably selective pressure of antibiotics and other cytotoxic substances have been hugely imposed by animal production.

The concept that acquisition of SCC*mec* element into the chromosome of methicillin-susceptible *S. aureus* (MSSA) generated MRSA strains has been well accepted. However, the origin of *mecA* had not been clarified as the absence of PBP2a homologue in its counterpart strain, MSSA. The *mecA* gene homologues have been detected in some coagulase-negative staphylococci (CoNS) species. *S. sciuri* and *S. vitulninus* have been reported to carry the *mecA*-like genes that showed 80% and 91% nucleotide identity to *mecA* gene, respectively.[48, 49] The ubiquitous of *mecA* gene homologues among *S. sciuri* species and the expression of homologue of PBP2a (87.8% amino acid sequence identity) suggested *S. sciuri* as the origin of *mecA* gene.[50, 51] However, these genes identified are lack of the constituents of the *mec* complex or SCC*mec*.[52] Recently, Tsubakishita *et al.* proposed that the animal-related staphylococcal species, *S. fleurettii* might be the origin of the *mecA* gene. This species is closely related to *S. sciuri* and *S. vitulninus,* which are rare among human but usually isolated from animals and food products of animal origin. The gene detected in *S. fleurettii* is identical to *mecA*. Although it was not associated with SCC*mec,* the gene was found on the chromosome that links with genes essential for growth of staphylococci.[52]

The beta-lactam resistance mediated by the expression of *mecA* gene has also been observed among CoNS species. Previous study of SCC*mec* types among 139 methicillin-resistant *S. epidemidis* (MRSE) strains revealed that the majority of MRSE carries SCC*mec* types IV (41%), followed by SCC*mec* types III (27%). Other SCC*mec* types including types V, I and II have also been observed among the minority of MRSE strains at 6%, 4%, and 4%, respectively.[41] In addition, several species of methicillin-resistant coagulase-negative staphylococci (MRCNS) have been found to carry novel and highly diverse types of SCC*mec* elements.[38] The process by which the SCC*mec* was acquired into the chromosome of staphylococci is unspecified due to a wide range of mobile genetic elements (MGEs) found in their genomes. However, the acquisition via transduction by one or other of numerous number of phages identified in staphylococci has been speculated.[53] It has been estimated by using eBURST algorithm (http://eburst.mlst.net) that the acquisition event of *mecA* happened around 20 times among *S. aureus* isolates with diverse genetic backgrounds (MLST sequence types).[45] Using the same method, higher numbers of *mecA* acquisition events, around [54], were also observed in a CoNS species, *S. epidemidis*.[41] Moreover, several non-*mec* SCC elements and ΨSCC (without or no functional *ccr*) carrying other genetic determinants have been described. These non-*mec* SCC was found to encode for traits that may essential for bacterial survival or pathogenesis, or examples, resistance to heavy metals (SCC*mer*) or fusidic acids (SCC*MSSA476*), capsule biosynthesis (SCC*cap*[1]) or aginine deiminase and oligopeptide permease (the arginine catabolic mobile element [ACME], ΨSCC*ACME*). Many of these have been identified in CoNS suggesting that CoNS may be responsible for assembling and dissemination of SCC elements, including SCC*mec*.[53] Furthermore, Price et al. studied the relatedness of 89 strains within the CC398 (from different host species from four continents) in order to understand their recent evolution by using the

whole genome sequencing (WGS) method.[22] The WGS phylogenetic results strongly supported the concept that the LA-MRSA CC398 originate as MSSA in human. The authors also explained that the switch of MSSA from humans to livestock was accompanied by the loss of phage-carried human virulence genes, followed by acquisition of SCC*mec* and tetracycline resistance. This finding provided a model of a bidirectional zoonotic exchange and underlined the protential public health risks of extensive use of antibiotics in food animal industry.[22]

Molecular typing of MRSA

As mentioned earlier, different categories of MRSA can be distinguished from each other based on their genotypes, phenotypes and epidemiology. However, during recent years their differences in the aspects of clinical epidemiology and antibiotic susceptibility have become increasingly blurred, thus various molecular typing methods have been increasingly used for characterization of newly emerging MRSA lineages.[11, 26] At presence, four molecular tools have been frequently used for typing of MRSA strains including pulsed field gel electrophoresis (PFGE) analysis, multi-sequence typing (MLST), staphylococcal protein A (*spa*) typing, and SCC*mec* typing.[18, 19]

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) is an agarose gel electrophoresis particularly used for separation of large DNA fragments that cannot be achieved by ordinary electrophoresis method. In PFGE, DNA fragments are separated by agarose gel electrophoresis in an electric field with an alternating voltage gradient. Macrorestriction of *S. aureus* using *SmaI* followed by PFGE analysis has been considered as the gold standard method for investigation of epidemic MRSA clones in hospitals. This technique has been proof to be useful for distinguishing CA-MRSA clone from HA-MRSA clones in the United States[54] and widely used in many countries. The patterns of digested chromosomal DNA of *S. aureus* strains are analyzed using software such as unweighted pair group matching analysis (UPGMA) according to Tenover *et al*.[55] This technique is one of the most discriminative methods for MRSA typing. However, dissimilar nomenclature of resulting patterns makes it difficult to compare results from different laboratories.[18] For example, the PFGE database in the United States classify major *S. aureus* clones as USA100, USA200, USA300, and so on, whilst nomenclature are different in the United Kingdom (EMRSA), Western Austraria (WA) and Canada (CMRSA).[11,18] Attempt for common nomenclature has been initiated many times but they have not yet successful. In spite of reproducibility pitfall, additional limitations of PFGE include cost of analysis and speed.[18]

Multilocus-sequence typing

Multilocus-sequence typing (MLST) is based on the DNA sequence analysis of fragments (approximately 450-500 bp) of seven *S. aureus* housekeeping genes, including *arcC*, *aroE, glpF, gmk, pta, tpi and yqiL.* A different nucleotide sequence of each housekeeping gene is assigned as a distinct allele. The alleles of the seven genes resulting in an allelic profile that defines the *S. aureus* lineage, designated sequence type (ST). For example, the MLST allelic profile 3-35-19-2-20-26-39 defined for the clonal lineage ST398 while another allelic profile, 3-3-1-1-1-1-10 defined for the clonal lineage ST9 (http://saureua.mlst.net). Furthermore, the clonal complex (CC) of related sequence types (ST) can be designated by using the algorithm based upon related sequence types or eBURST (http://eburst.mlst.net). Generally, the allelic profile of each ST that has at least identical 5 housekeeping loci can be designated into the same CC. In contrast with PFGE, MLST is an unambiguous procedure for characterizing bacterial isolates using the universal nomenclature.[56] Nevertheless, this method is expensive, laborious and time consuming.[18]

Staphylococcal protein A typing

Staphylococcal protein A (*spa*) typing is the sequence-based method specifically analyzes the polymorphic variable-number tandem repeat (VNTR) region or "X" region (approximately 24 bp) of

staphylococcal protein A. The duplication, deletion, and rearrangement of *spa* gene in each *S. aureus* linage generate a typical *spa* gene. Distinct repeat number of "X" region in *spa* gene, presumptively, yield more than 10,000 unique patterns named as "*spa* types" or "t" (http://spaserver.ridom.de). The discriminative power of this technique is higher than MLST but lower than PFGE.[18] Cluster analysis of *spa* typing data are also possible by the use of the algorithm based upon repeat pattern (BURP) into StaphType software.[18] Since the *spa* typing of MRSA strains has been demonstrated to be well correlated with MLST results, therefore, it can be used to infer the clonal complex.[11] Compared with MLST, *spa* typing, involving analysis of only one locus, is more cost effective, less time-consuming and less laborious.[18]

SCC*mec* typing

The SCC*mec* typing is based on the various combinations of differences of each *mec* complex and *ccr* genes. Thus, this method can be used to classify distinct allotypes of the SCC*mec* horizontally acquired into MRSA strains while other molecular typing methods (PFGE, MLST, and *spa* typing) describe the genetic background of *S. aureus*.[57] The SCC*mec* types can be identified using PCR-based techniques developed by groups of researchers.[58-63] Since the novel types of the SCC*mec* elements have continued to be recognized, [46, 47, 64] therefore, limit the use of previously reported protocols. Currently, only the multiplex PCR method reported by Kondo, *et al*., can be useful for characterization of most SCC*mec* types reported including type-IX SCC*mec* recently emerged in Thailand.[46, 64-66] The PCR-based SCC*mec* typing may not be practical to use in routine hospital laboratory as it is associated with many SCC*mec* loci. However, it allows universal description of the MRSA clones and usually can preliminary categorize the MRSA isolates of different origins. It is well documented that the CA-MRSA and LA-MRSA usually are associated with SCC*mec* types IV and V where as the HA-MRSA commonly carry types I, II and III SCC*mec* elements. In addition, increased resolution of this method by SCC*mec* subtyping of J regions, might provide insight into the clonal backgrounds of MRSA isolates, as in the case of SCC*mec* types IV.[11] To date, nomenclature of MRSA relies on the MLST sequence type (ST) or clonal complex (CC) and the SCC*mec* types e.g. ST8-IVc, ST9-IX, ST398-IV, etc.

Epidemiology of CA-MRSA

A numbers of CA-MRSA clones have spread worldwide since the 1990s, currently; more than 20 distinct genetic lineages of CA-MRSA have been reported. This is greatly higher than HA-MRSA lineages, suggesting that more MRSA lineages have evolved to CA-MRSA compared with HA-MRSA.[11] The majority of the CA-MRSA isolates harbor SCC*mec* type IV, V or VII, however, several studies have observed CA-MRSA isolates that harbor SCC*mec* type I, II or III.[67, 68] At present, five predominant PVL-positive CA-MRSA clones have disseminated around the world including ST1-IV (WA-1, USA400), ST8-IV (USA300), ST30-IV (South West Pacific clone), ST59-V (Taiwan clone), and ST80-IV (European clone).[13] Among these clones, ST8-IV and ST30-IV may be considered pandemic.[11]

Cases of ST8-IV or USA300 cases were first recognized in a college football team in Penisynvania, the United States. This clone have been circulating in the community among particular health risk groups such as military men, prisoners, athletes and intravenous drug users and afterward become primary cause of skin and soft tissue infection among general population in the United States. USA300 harbors a SCC*mec*-like element named "arginine catabolic mobile element (ACME)" that have not been detected in other CA-MRSA clones. The ACME encodes for an arginine deiminase pathway and an oligopeptide permease system; thus, it has been suggested to be involved in growth and survival as well as its virulence in pathogenicity.[33, 69] This clone has replaced the USA400 (ST1-IV) that dominated earlier in healthcare facilities. Previously, a study by the emergency departments in 11 cities of the United States concluded that 78% of the isolates were MRSA. Of these, 98% were associated with the CA-MRSA USA300 clone.[70] Recently, high frequencies of multidrug-resistant (MDR) USA300

strains harboring conjugative plasmids conferring high-level mupirocin resistance have been reported in San Francisco.[71] The ST8-IV has also been increasingly isolated from hospitals in Taiwan and now exceeded those of HA-MRSA clone. Cases of USA300 outside the United States have been documented in many countries including Canada (CMRSA-10), Japan, Australia, as well as some of the European countries.[11] These evidences have posed major concern to public health worldwide.[11] In 2006, the USA300-like strains designated "USA300-LV" (Latin American variant), the AMCE-negative SCC*mec* IVc circulating in South America, Europe and Australia have emerged in both community and hospital in Colombia.[72]

ST30-IV or South West Pacific clone (WPS) currently predominate around the world including Asia. This clone was the descendent of the pandemic phage-type 80/81 penicillin-resistant *S. aureus* or ST30 MSSA.[73] This *pvl*-positive ST30 clone disappeared after the emergence of the first MRSA clone in the 1960s and re-emerged in the community as ST30-IV MRSA. In addition, it was suggested that this clone shares the same ancestor (ST30 MSSA) with ST36-II HA-MRSA or epidemic EMRSA-16 in the United Kingdom. ST30-IV was first reported in 2003 in Greece, the country with the highest cases of CA-MRSA infection in Europe. Although ST30-IV strains often cause severe diseases, however, they are generally susceptible to more antibiotics than other CA-MRSA strains.[11]

The epidemiology of MRSA, especially CA-MRSA, in Asia is relatively limited. However, reports of CA-MRSA cases from Taiwan have been increased during the past decade. In Taiwan, significant increase of CA-MRSA carriage and infection among healthy subjects was observed. More than 50% of pediatric *S. aureus* infections associated with community were caused by MRSA.[74] The rate of healthy adults carrying *S. aureus* is around 30–50%. In 2000, the MRSA prevalent rate was 3–83% of all *S. aureus* isolated in 12 major hospitals in Taiwan. The most frequently isolated CA-MRSA clones were ST59-IV and ST59-VT. The first one is PVL-negative and usually isolated from healthy carriers of MRSA. However, the latter carries a variant of type-V SSC*mec* called "SCC*mec* type VT" and *pvl* genes. This clone is more prevalent among patients.[74] These clones are also the most prevalent CA-MRSA strains in China, and several other Asian countries, but are also found in Europe, Australia, and the United States (USA1000).[75]

As well as in many countries in South East Asia, little is known about MRSA carriage and CA-MRSA infection among Thai population. In Thailand, the study of the antibiotic susceptibility of common community- and hospital-associated bacteria in Thailand (from years 2002-2003) in 24 hospitals was first recognized in 2005. This revealed that the community-associated bacteria (CAB) accounted for 54.9% of a total of 9,091 isolates of target bacteria (eight common bacterial pathogens including *S. aureus*), which is higher than HA-bacteria (45.1%). It was found that CA-MRSA strains were more susceptible to antimicrobials compared to hospital acquired strains. However, decreased susceptibility to antimicrobials was found in all CAB tested indicating the widespread resistant bacteria to the community.[76] The prevalence of CA-MRSA infections was first reported in 2006. The rate of MRSA-infected patients at Siriraj Hospital was 41.5% (186 of 488 studied patients). However, the CA-MRSA strains were found to be rare (0.9% of total MRSA isolates), only three isolates from two patients were detected.[77] In 2010, the SCC*mec* types, MLST and antimicrobial susceptibilities of HA-MRSA isolated from 237 patients treated at Srinagarind Hospital, Khonkan, Thailand, between September 2002 and August 2003, have been investigated. Of the 81 isolates tested,[76] MRSA isolates were found to carry SCC*mec* type III and two isolates were found to carry type-II SCC*mec*. Of these, non-*mecA* MRSA strains was detected in three isolates and 67 of 78 isolates carried the mercury-resistant operon. All MRSA isolates were susceptible to vancomycin, but only 0.4% to 8.9% was susceptible to the remaining antimicrobial agents.[78] In addition, the first study of MRSA nasal colonization among healthy young adults in 2011, suggesting that MRSA was present in the Thai community. Whereas 15% (30 of 200) were nasal carriages of MSSA, only 1% of

university students carried MRSA strains. However, two MRSA isolates were found to carry type-II SCC*mec*, which are not any of SCC*mec* elements typically acquired by CA-MRSA strains. All MRSA isolates were susceptible to non-bata-lactam antibiotics.[79]

A recent survey, nevertheless, reported the prevalent rate of CA-MRSA in Asian countries including Thailand from September 2004–August 2006.[80] This prospective surveillance study conducted by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) with 17 participated hospitals in Asian countries, including two hospitals from Thailand. The results showed that MRSA infections in the community have been increasing in Asian countries. Of 4,117 isolates from patients, MRSA accounted for 25.5% of community-associated infections by *S. aureus* and 67.4% of hospital-associated infections. The most prevalent genotypes of CA-MRSA isolates were ST59-IV-t437 (*spa* type), ST30-IV-t019 and ST72-IV-t324. The highest prevalence of CA-MRSA and HA-MRSA was observed in Sri Lanka at 38.8% and 86.5% of total *S. aureus* isolates, respectively.

High rates of CA-and HA-MRSA also found in Taiwan and Vietnam, where the CA-MRSA rate was more than 30% and HA-MRSA rate was more than 65%. The relatively low rate of CA-MRSA isolates among Thai patients was found at approximately 2.5% (3 of 122) of all *S. aureus* identified, in contrast with 57% (180 of 316) prevalence rate of HA-MRSA. Interestingly, CA-MRSA isolates were classified into two SCC*mec* types. Of three CA-MRSA isolates, two were SCC*mec* type III (66.7%) and one was SCC*mec* type II (33.3%). This was similar to what found among HA-MRSA isolates from Thailand that comprised of only two types of SCC*mec*. Of 29 HA-MRSA isolates, 14 were SCC*mec* type III (82.8%) and 5 were SCC*mec* type II (17.2%). The *spa* type t037 appeared to predominate among SCC*mec* type III MRSA from Thailand. However, *spa* type t654 were also identified, at a lower rate, in both CA- and HA-MRSA stains containing SCC*mec* type III. In addition, one SCC*mec* type II CA-MRSA isolate had *spa* type t2879. The ST239-III-t037 clone was found to be the most prevalent clone among CA-and HA-MRSA isolates from Thailand. Intriguingly, ST239 was previously established as nosocomial MRSA clones was also detected in the CA-MRSA isolates.[80] This evidence was also observed in other Asian countries that ST5 clone was found in CA-MRSA isolates whereas MRSA clones such as ST59, ST30 and ST72 were isolated from patients with HA infections. These data imply the spread of various MRSA clones between the community and hospitals.[80] Furthermore, a recent work by Nickerson *et al.* in 2011 reported that ST834 clone were predominant (91%) among Cambodian children. ST834 was identified previously as a cause of CA-MRSA infection in the same population. Other ST clones including ST121, ST188, ST45 and ST9 was also identified, at lower rates, around 1 to 3% of all 93 MRSA isolates. Although, a relatively low rate of MRSA carriers was found at 3.5% (87 of 2,485 children who came to the outpatient department, the Angkor Hospital for Children, Siem Reap), further characterization of ST834 should be carried out to elucidate its competitive advantage over other ST clones found among Cambodian population.[81]

Emergence of LA-MRSA in Thailand

The emergence of human LA-MRSA infections in all continents of the world has also been reported and the bi-directional transmission of LA-MRSA between livestock and human has recently been established.[22] The CC398 is the most prevalent lineage among LA-MRSA isolated from pigs followed by CC9.[82] However, other clones including CC1, CC5, CC8, CC9, CC30, and CC97 have also been reported.[25, 83, 84] Unlike CA-MRSA, LA-MRSA clones have unique molecular genetic trait and could not be typed by PFGE method using *Sma*I *restriction*.[26, 85]

Nevertheless, their genotypes can be examined by using other molecular tools. In addition, most of them carry genes that confer co-resistance to non-beta-lactam antibiotics and heavy metals, but their carriage of genes encoding toxin such as PVL and enterotoxins have been reported to be rare.[26] The latest published whole genome sequences of *S. aureus* CC398 revealed heterogeneity between human and livestock-associated lineages.

This has alleviated differentiation of *S. aureus* CC398 in human and animals based on the presence or absence of specific resistance and virulence-related genes. The CC398 stains typically carry *tet*(M) conferring tetracycline resistance, but not *pvl* genes encoding for PVL toxin, generally found in CA-MRSA strains.[22]

Recently, LA-MRSA isolates originally from Thailand have been discovered. The first literature documented the nasal colonization of MRSA of Thai veterinarian participated an International conference in Denmark in 2007.[86] Only one isolate of MRSA was recovered and it was identified as *spa* type t034 and ST398, a typical clone of LA-MRSA. In 2011, Anukool, *et al.* firstly reported LA-MRSA isolated from pigs in Thailand.[64] Our survey of MRSA in pig farms located in Lamphun Province, Northern Thailand, discovered five MRSA isolates from four nasal swabs and one rectal swab of four weaning pigs. All of these were characterized as ST9-t337, which are closely related to pig MRSA strains reported in other Asian countries including Hong Kong (ST9-t899)[87] and Malaysia (ST9-t899)[64] as well as that latest reported in Taiwan (ST9-t899-V).[88] The ST9 strains were classified as type-1 *ccr* complex (*ccr*A1B1) and *mec* class C2 (IS*431mecA*-Δ*mecRI*-IS*431*), which is now known as the novel SCC*mec* type IX (Table 1, Figure 2).[46] Interestingly, the J regions of type-IX SCC*mec* element carries genes and operons associated with resistance of many heavy metals, including cadmium, copper and arsenate (Figure 2). The 18-kb J1 region of type-IX SCC*mec* harbors a *cad*DX operon, a *cop*B gene, and two arsenate resistance operons, *arsRBC* and *arsDARBC*. It was also found that all 20 open reading frames (ORFs) in the J1 region were highly homologous to the SCC*mec* elements in *S. haemolyticus* JCSC1435, with nucleotide identities of 83.8 to 100%.[46]

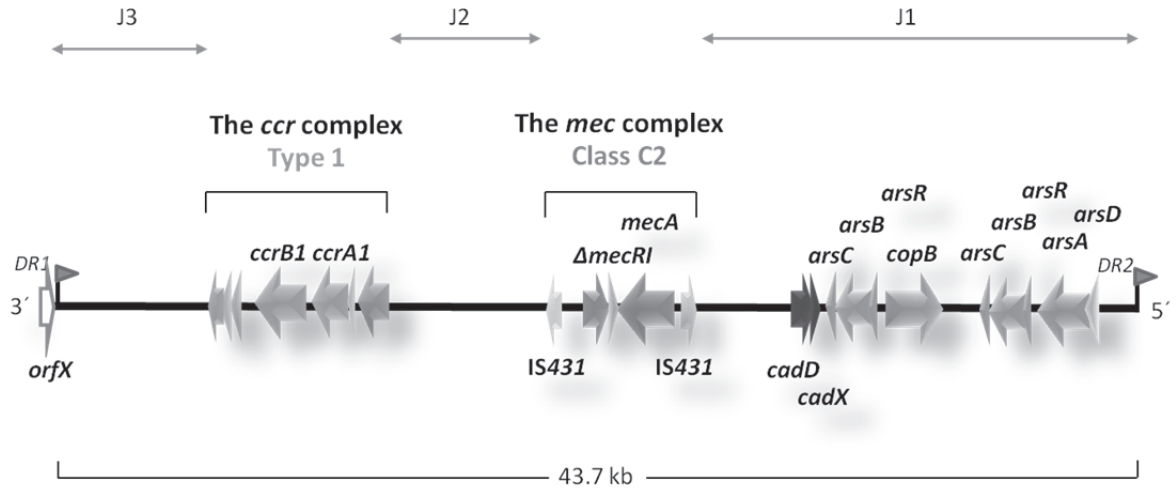

Figure 2 The structure of the SCC*mec* type IX identified in CC398 MRSA strains (adapted from Li et al., 2011). [46] The SCC*mec* type IX carries type 1 of the *ccr* complex and class C2 of the *mec* complex. J1, J2 and J3 stand for the junkyard regions 1, 2 and 3, respectively. The 18-kb J1 region harbors various genes and operons conferring resistance to heavy metals, including copper (*copB*), cadmium (*cadD, cadX*) and arsenate (*arsRBC* and *arsDARBC*).[46] DR, direct repeat. Red arrow heads indicate the locations of integration site sequences (ISS) for the SCC element.

In 2012, two studies, one conducted in the north and another in the middle part of Thailand, reported the discovery of the CC9-t337-IX clones in pig and pork in Thailand.[65, 66] Larsen *et al.* isolated MRSA strains from pig nasal swabs from three (in the same village) out of 30 farms located in Chiang Mai Province. All isolates were identified as ST9-t337-IX LA-MRSA.[65] The screening of MRSA in pigs and pork at Samut Songkhram Province by Vestergaard *et al.* revealed that all 11 MRSA isolates belong to single *spa* type, t337. Three sequence types were found in isolates from pork samples including ST2136 (n=3), ST9 (n=1) and ST2278 (n=1). Two sequence types were found in isolates from pigs including ST2136 (n=5) and ST9 (n=1). However, all of these sequence types are classified as the same clonal complex, CC9.[66] To date, the carriage of SCC*mec* type IX in pigs, pork and human have only observed in Thailand. These data suggest the presence and plausible spread of a unique LA-MRSA lineage in Thailand. Although, these isolates are PVL-negative, which is typical in LA-MRSA, some of them were found to harbor other toxin genes that may increase their risk as human infection. Furthermore, the highly antibiotic resistance profiles observed among these strains are of public health concern of their spread in the community.

Conclusion

In Thailand, as well as many resource-limited countries, the knowledge of CA- and LA-MRSA is still limited. Information of CA-MRSA has been dominated by extensive publications of USA400 and USA300 clones from the United States whilst the majority of LA-MRSA information, particularly, CC398 clone harboring SCC*mec* type IV and V, have arrived from European countries. Recent reports of human and animal colonization and contamination of animal food product with CA- and LA-MRSA clones in Thailand should stimulate attention to this important human pathogen which is possibly disseminating in the community. Extensive research to investigate the prevalence and genetic backgrounds of MRSA lineages in Thailand have to be conducted. The medical technologists will play a crucial role to promptly identify these pathogens. Although, the definition of different MRSA categories has been described by the CDC, the classification of these groups based on their epidemiological data is almost inapplicable. Current molecular methods used to distinguish MRSA clones are still expensive and not suitable for routine laboratory in hospital. Rapid and cost effective technique for detection and identification of MRSA lineages is required to lessen public health and economic burdens caused by these highly adapted bacteria, particularly, in resource-limited countries like Thailand.

Interestingly, the localization of novel SCC*mec* type IX in ST9 and ST398 LA-MRSA isolates so far has been found only in Thailand. This might suggest recent acquisition of type-IX SCC*mec* element by these lineages. The particular CC9-t337-IX clone found in pigs and pork might have evolved for sometimes to survive under particular environmental conditions. Selective pressure and co-selection of resistance determinants might be accounted for the high antimicrobial resistance profile among these isolates. Thus, it may reflect the superfluous usage of antimicrobial treatment and prophylaxis as well as a high contamination of heavy metals in animal feeds used in animal food production in Thailand. Future appropriate control and management of these substances used in livestock must be implemented to avoid development of highly resistant bacteria, especially those that have been known as potential pathogens in human and animals. The acquisition of virulent factors such as PVL of the MDR LA-MRSA strains, which are now pandemic and widely distributed in animal reservoirs, would result in life-threatening diseases in human. Unawareness of CA- and LA-MRSA spread may bring about unmanageable public health crisis. Active surveillances of MRSA infection and colonization, both in human and animals, and future control measures are needed to prevent the (now occurring?) epidemic spread of CA- and LA-MRSA in Thailand.

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