REVIEW ARTICLE

CHARACTERISTICS, DETECTION AND TYPING METHODS OF COMMUNITY ACQUIRED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (CA-MRSA) – A REVIEW

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ABSTRACT

Staphylococcus aureus are gram positive cocci which colonize the skin and mucous membranes particularly the anterior nares. Prevalence of nosocomial infections associated with methicillin resistant S. aureus have been reported in hospitals (HA-MRSA) for over five decades. Recently, community-acquired MRSA (CA-MRSA) has emerged as a cause of skin and soft tissue infections in healthy individuals. These strains are sensitive to antimicrobials, carry genes for Panton-Valentine leukocidin (PVL) toxin and belong to the staphylococcal cassette chromosome (SCC) mec type IV or V. The suspected mode of transmission involves close contact with carriers leading to skin or nasal colonization that result in subsequent active infection. Molecular typing is used to determine the mode of transmission of CA-MRSA in the community. General typing methods such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) and specific methods for Staphylococci such as SCCmec typing and spa typing have the capability to characterize bacterial chromosomes and mobile genetic elements. Combination of these molecular typing methods is necessary as each method has its own advantages with respect to discriminatory power, rapidity, cost effectiveness, reproducibility, and ease of performance.

Key words: CA-MRSA, prevalence, transmission, PVL toxin, SCCmec, spa typing, PFGE, MLST

INTRODUCTION

Staphylococcus aureus is a causative agent of both suppurative diseases such as folliculitis, furuncle, carbuncle, impetigo and metastatic infections such as osteomyelitis, septic arthritis, and pneumonia. *S. aureus* possesses toxins which are responsible for food poisoning and toxic shock syndrome. Bacteremia and endocarditis are examples of staphylococcal infections that can lead to fatality[1]. Penicillin was effective to treat staphylococcal infections until penicillin-resistant *S. aureus* were detected[2]. Methicillin was introduced as a semisynthetic drug to ward off the penicillin resistant strains. Methicillin-Resistant *S. aureus* (MRSA) strains emerged after two years of methicillin usage and they were primarily associated with nosocomial infections and termed as hospital acquired-MRSA (HA-MRSA)[3,4]. In the early 1990s, MRSA infections indicated an altered trend in which individuals without healthcare associated risk factors developed infections. These infections are believed to be acquired in the community and caused by strains referred as Community-acquired MRSA (CA-MRSA).

Definition of CA-MRSA

The definition of CA-MRSA infection has been proposed by Centers for Disease Control and Prevention (CDC). A CA-MRSA must be isolated from an outpatient or isolated within 48-72 hours of hospital admission. In addition, the patient must not have the history of previous hospitalization, antimicrobial therapy within past 6 months, surgery and dialysis, medical devices or catheters that are permanently attached through the patient's skin, and family members exposed to healthcare settings[5-8].

Prevalence of CA-MRSA:

The first CA-MRSA cases were reported from the indigenous Aboriginal population from Western Australia[9]. In 1997, CDC reported a CA-MRSA outbreak among four pediatric patients in Minnesota and North Dakota, USA, all suffered from pneumonia. The MRSA isolates obtained from all these patients were susceptible to antibiotics other than beta-lactams[10]. CA-MRSA infection also reported among native American villagers and the risk factor of infection was attributed to sharing contaminated saunas and antibiotic treatment[11]. High prevalence of CA-MRSA skin and soft tissue

infections among Pacific Islanders was also reported in Hawaii [12].

The prevalence of MRSA in the community and the hospital settings was studied in eight Asian countries from September 2004 to August 2006 that participated in ANSORP, the Asian Network for Surveillance of Resistant Pathogens. The overall HA-MRSA and CA-MRSA rates were 67.4% and 25.5% respectively. The highest prevalence of CA-MRSA was reported in Sri Lanka (38.8%), Taiwan (34.8%), Vietnam (30.1%) and Philippines (30.1%). The CA-MRSA prevalence in Korea, Hong Kong, India, and Thailand were 15.6%, 8.5%, 4.3%, and 2.5% respectively. The CA-MRSA were predominantly isolated from pus samples (70.5%) and less likely from blood (9.1%), sputum (8.6%), and urine (4.3%)[13,14]. The first CA-MRSA case in Malaysia was documented in 2008 and the strains isolated since then were reported to carry SCC*mec* IV or V and Panton-Valentine leukocidin (PVL) toxin genes[15-18]. The preliminary alert organism surveillance data of Hospital Wanita Dan Kanak-Kanak Sabah (HWKKS), Sabah, Malaysia showed 35 (70%) and 49 (86%) CA-MRSA cases in 2014 and 2015 respectively of the total MRSA cases showing an increasing trend in recent years (unpublished data). The exact route of transmission has not been identified in the above cases. However, close contact with carriers leading to skin or nasal colonization and subsequent active infection might be considered as the source of infection.

CA-MRSA associated infections

Younger patients with no healthcare associated risk factors are more likely to suffer from CA-MRSA infections. The common clinical symptoms of the CA-MRSA among children were abscess, cellulitis, and pneumonia[6,19]. The incidences of pyomyositis and myositis cases caused by CA-MRSA strain, USA300 have been shown to have increased in a 5 years period in Texas Children's Hospital[20]. CA-MRSA infections were also shown to be associated with Waterhouse-Friderichsen syndrome, necrotizing fasciitis, purpura fulminans etc[21-23].

Characteristics of CA-MRSA isolates

CA-MRSA strains are found to be smaller in size compared to HA-MRSA due to their less metabolic burden on protein synthesis during replication. They have a faster growth rate which facilitates successful colonization in humans. The CA-MRSA isolates were shown to be more susceptible to antimicrobials compared to nosocomial isolates[24]. Molecular analysis of CA-MRSA isolates from different regions including USA, France, Switzerland, New Zealand, Australia, and Western Samoa revealed that they share same genetic characteristics, such as harboring SCC*mec*IV or V element and PVL toxin[25]. It is believed that *S. aureus* strain was initially infected by a phage harboring PVL genes (lukS-PV and lukF-PV) and integrated into the chromosome and became a prophage. Later, horizontal transfer and insertion of SCC*mec* element which consists of *mec*A gene into the chromosome led to the existence of CA-MRSA[26].

1.SCC*mec* element:Staphylococcal cassette chromosome (SCC) elements are genomic islands that serve as machineries to capture foreign DNA segments in order to survive in harsh environments. SCC*mec* element was recognized after two recombinase genes, *ccr*A and *ccr*B, encoding recombinase A and B respectively were identified while nucleotide sequencing of the entire *mec*DNA of MRSA strain, N315. The recombinase A and B are responsible for the excision and integration of SCC*mec* element into *S. aureus* chromosome[27,28]. SCC*mec* element encodes methicillin-resistance which is a modified penicillin-binding protein. Based on International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), SCC*mec* element consists of *ccr* gene complex and *mec* gene complex. The SCC*mec* types are determined by a combination of *ccr* gene complex and *mec* gene complex. The *ccr* gene complex consists of *ccr* genes and surrounding open reading frames with unknown function. The *mec* gene complex consists of *mec*A gene, *mec*I and *mec*R1 gene (regulatory genes), and 20 to 45 kb of *mec*-associated DNA (insertion sequences). SCC*mec* typing shows that the CA-MRSA strains generally carry SCC*mec*IV or V elements where SCC*mec*IV possesses Type 2 *ccr* gene and Class B *mec* gene complex while SCC*mec*V possesses Type 5 *ccr* gene and Class C *mec* gene complex[24,29]. The SCC*mec*IV element is further divided into several subtypes based on the differences in J1 region found between the right chromosomal junction and the *ccr* complex[30].

2.Panton-Valentine leukocidin (PVL) toxin: Panton-Valentine leukocidin (PVL) is a cytotoxin that causes leukocyte destruction and tissue necrosis and has become one of the constant markers for CA-MRSA. PVL toxin is composed of two classes of proteins, class F (LukF-PV) and class S (LukS-PV), they bind together into a heptamer and form pores on the polymorphonuclear leukocytes membranes[26].

Previous findings show that *S. aureus* isolates that carry PVL toxin genes were linked with furunculosis, community-associated pneumonia, acute hematogenous osteomyelitis, myositis and pyomyositis particularly in younger patients[31,32]. Occurrence of sepsis and coagulopathy caused by

PVL positive CA-MRSA strains were reported among adolescents[33,34]. Patients who were infected with the PVL positive CA-MRSA strains required more surgical drainage procedures and needed more attention in ICU[20]. Pneumonia associated with the PVL positive *S. aureus* was considered lethal because of the lower survival rate after hospitalization[32].

Staphylococcus aureus colonizes particularly anterior nares

S. aureus is the normal microflora of the human skin and colonizes particularly the nose, axillae, perineum, back of the wrist and toewebs[35-37]. The colonization rates were reported to be 9.2%, 9.9%, and 20.7% in vagina, labia minora, and anterior nares respectively showing that the anterior nares were more likely to be colonized with a higher percentage of this bacterium[38]. The presence of large apocrine glands in anterior nares and perineum gives a suitable environment for staphylococci to live[39]. Moreover, nasal vestibule (vestibule nasi) or anterior nares are lined with keratinized, stratified squamous epithelium, sweat glands, and sebaceous glands and free from the host defenses thus serves as a suitable spot for staphylococci to reside. Nasal carriage rate was shown to be higher when the nasal swab samples were obtained from the nasal vestibule compared with the mucous membrane of the nasal fossa[40].

Colonization *S. aureus* is divided into 3 types; persistent carrier, intermittent carrier, and noncarrier. Persistent carriers consist of 20% in the general population and they normally carry only one type of strain. They might gain the same staphylococcus strain from the other body sites or from the family contacts[41]. Sixty percent of the population comprised of intermittent carriers where a certain type of strain colonizes them temporarily before replaced by another type of strain. Only 20% population is categorized as noncarriers[42]. CA-MRSA colonization has been reported among healthy children attending day care centers, kindergartens and pre-schools in Korea, Hong Kong and Taiwan[43-45].

Bacterial and host factors influence Staphylococcus colonization

The interaction between the host cell membrane and the bacterial cell wall component is essential for successful colonization which is influenced by several factors. Bacterial extracellular structures facilitate the bacterial adherence to the nasal mucosal cells. Fibronectin-binding protein of *S. aureus* functions by binding to the human fibronectin while teichoic acid was shown to inhibit the adherence of staphylococci to the host epithelial cell[46,47]. Bacterial interference by decolonization of *S. aureus* in newborns shown to significantly increase colonization by gram-negative bacilli[48].

S. aureus shows high affinity and binding to the nasal mucosal cells of persistent carriers as compared to noncarriers[49]. Host factors such as age, race, histocompatibility antigens (HLA), nasal abnormalities, bacterial/viral infections and sex hormones were shown to be associated with *S. aureus* colonization. Children are more inclined to be persistent carriers compared to adults. The persistence of *S. aureus* carriage was also observed among people between 10 to 20 years old. Adults are generally intermittent carriers of *S. aureus*[50]. Caucasians have a higher rate of *S. aureus* nasal colonization compared to black Africans[51]. Individuals with HLA-DR3 type was linked with *S. aureus* nasal carriage, while individuals with HLA-DR2, HLA-DRl and HLA-Bw35 were less likely to be carriers[52]. Healthy people with nasal abnormalities such as damaged turbinate and deviated septum display high rates of *S. aureus* nasal colonization[40]. People who were affected by a viral infection in the upper respiratory tract display greater adherence of *S. aureus* due to alteration of the nasal mucosal cells[53]. Previous hospitalization, previous antimicrobial treatment with cotrimoxazole, and lower CD4 counts in HIV patients are shown to be the risk factors of MRSA colonization among tuberculosis patients[54]. Lastly, women with high karyopyknotic index (KI) or estrogen level are prone to *S. aureus* nasal carriage[55].

Transmission of CA-MRSA:

Familial transmission occurs when two or more members of a family who live in the same postal address are colonized with MRSA strains that have similar genotype. CA-MRSA disseminates from children to parents or vice versa. Transmission is also possible between families as it has been shown that two families who lived in the same neighbourhood were found to be colonized with similar CA-MRSA strains[56-58]. Dissemination of CA-MRSA strain in the community setting encircling 26 households was reported in Denmark. Dissemination of CA-MRSA also occurs from the workplace to the household or vice versa. CA-MRSA strains might also disseminate from the other countries[59]. The following five risk factors for CA-MRSA (5 C's) transmission are proposed by CDC. They are cleanliness (poor hygiene), compromised skin integrity, frequent skin contact (close contact), contaminated surfaces or shared items, and crowding or frequent antibiotic usage or overuse of antibiotics[60].

The multidrug resistant USA300 clone in homosexual men was reported in San Francisco causing skin and

soft tissue infection (SSTI), including folliculitis, cellulitis, and abscesses[61]. CA-MRSA skin and soft tissue infection was reported in a college football team and the risk factors were shown to be sharing towels, bar soaps and delaying the treatment of cuts and abrasions[62]. CA-MRSA cases were reported among prisoners in USA and the risk factors involved were long period of stay, history of antibiotic use, crowding, unhygienic condition, and comorbidities. The military population also shared similar risk factors of CA-MRSA infections and the additional risk factors included were sharing crowded barracks and physical trauma associated with the recruitment training[63].

CA-MRSA infections were reported in two child centers involving an index patient with pneumonia and preseptal cellulitis and colonization among other children who were MRSA asymptomatic carriers which was confirmed by similar PFGE pattern. The risk factor of the MRSA colonization was due to two to three courses of oral antibiotic consumption and closed contact between children[64].

CA-MRSA has started to spread from the community to hospitals, neonatal intensive care units (NICUs), nurseries, maternity settings and among healthcare workers[13,65-68]. CA-MRSA carriage among newborns without risk factors was reported in the UK healthcare setting[69]. A clinical case of CA-MRSA transmission from father to infant was reported in the intensive care unit in Saudi Arabia[70]. CA-MRSA colonization was also observed among preclinical and clinical students[68]. Predisposing factors for *S. aureus* colonization were shown to be smoking, chronic sinusitis, usage of antibiotics in the past 3 months, and utilization of the hospital entrance[45,71,72].

Methods for detecting CA-MRSA transmission

Screening or active surveillance of CA-MRSA is conducted by collecting the nasal swab sample from an individual who is not presenting with any symptoms of disease. The sample is then processed in the laboratory to determine whether the individual is a MRSA asymptomatic carrier[73]. The implementation of rapid diagnostic methods with high sensitivity is crucial to detect the colonization[74]. Typing is necessary for epidemiological investigations to determine the source and mode of transmission of CA-MRSA strains in the community[75]. Currently, there is no single ideal typing method available and a combination of different typing methods has been used to understand the transmission of MRSA in the community[76,77].

Conventional methods: Culture and antibiotic susceptibility testing are used for the identification of CA-MRSA. Introduction of chromogenic agar excludes the requirement of subculture from the primary isolation plate and biochemical tests for MRSA identification thus help to minimize the time. The chromogenic agar such as Chromogenic MRSA/Denim Blue agar, oxacillin resistance screening agar base, MRSA Select CHROM agar, MRSA Ident agar are more expensive than the media used in conventional microbiological methods[78]. The antibiotic susceptibility tests show that the MRSA isolates identified from the patients without risk factors were susceptible to erythromycin, clindamycin, gentamicin, and trimethoprim-sulfamethoxazole[6]. Rapid detection of ciprofloxacin-resistant MRSA is possible within five hours by using BacLite *Rapid* MRSA (Acolyte Biomedica, UK) where ATP production is quantified by bioluminescence[79]. The above phenotypic methods have limited discriminatory power and poor typeability.

***mec*A gene PCR:** Detection of MRSA colonization among patients was greatly improved when PCR is performed for *mecA* gene[80]. The time allocated for the detection is reduced from four days to one day. The qMRSA (multiplexed-real time PCR) detects *mec*A and *fem*A genes from *S. aureus*, and only *fem*A gene from *S. epidermidis* and thus the sample which contained MRSA and *S. epidermidis* can be easily distinguished[81].

Molecular typing methods

Genetic knowledge has been applied in the clinical management in order to determine the source of infection. Molecular characteristics of the bacterial isolates found in patients and healthy individuals, especially the family contacts are compared. If the molecular characteristics of the bacterial isolates are similar, then the transmission of the single strain was found to have occurred[82]. Various molecular typing methods have been used such as pulse-field gel electrophoresis (PFGE), multi locus sequence typing **(**MLST), SCC*mec* typing and spa typing[83,84].

Pulse field gel electrophoresis (PFGE): PFGE is considered as the 'gold standard' among molecular typing methods for *S. aureus* due to its excellent discriminatory power and intra-laboratory reproducibility. Restriction endonucleases spot the specific sites on the bacterial chromosome and digest the bacterial DNA into large DNA fragments. These large DNA fragments are then separated by gel electrophoresis by electric current which is periodically switched in different directions. If the bacterial isolates display similar band patterns in the gel, then the bacterial isolates are considered genetically related[76,77,85]. PFGE clearly

discriminated MRSA and methicillin susceptible S. *aureus* (MSSA) isolates that differed by just two bands in pediatric patients with pneumonia[86]. The method is technically demanding, labour-intensive, time-consuming, with limited interlaboratory portability and it may lack the resolution power to distinguish bands of nearly identical size[76, 87].

Multilocus sequence typing (MLST): MLST involves PCR amplification and sequencing of seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, and yqiL). The sequences at each of the seven *loci* are compared with all the known alleles at that *locus,* and a number representing a previously described allele or a new one is assigned to the *locus*. For a given isolate, alleles present at each gene position are combined into an allelic profile and assigned a sequence type (ST) designation[77]. Closely related isolates have identical STs, or STs that differ at a few *loci*, whereas unrelated isolates have unrelated STs. PCR is simple and cheap and MLST data has interlaboratory portability with standard nomenclature. However, the MLST has only moderate discriminatory power, too labor-intensive, and time consuming[24,76,87].

SCC*mec* typing by multiplex PCR: SCC*mec* typing of the MRSA isolates is based on the combination of *mec* gene complex and *ccr* gene complex. PCR amplification is performed with specific primers which target the oligonucleotide sequences of the SCC*mec* elements[88]. SCC*mec* typing has been improved by the introduction of novel multiplex PCR primers that are specific for SCC*mec* subtypes which become cost and time-efficient[89]. The methods are relatively straight forward, simple to perform, and inexpensive. However, it can be less useful in investigating local outbreaks, as the SCC*mec* elements are generally stable[77].

***Staphylococcus aureus* protein A (*spa*) typing:** Protein A is the virulent factor in *S. aureus* which binds nonspecifically to the Fc region of the antibody. The hypervariable X region of *spa* gene which encodes for protein A become one of the steadfast molecular typing methods in certain countries such as Belgium and Germany for MRSA active surveillance because of its rapidity and high throughput[90,91]. Initially, the X region of the spa gene consists of different short sequence repeat regions (SSRRs) is amplified by PCR followed by sequencing. Each SSRR contains about 24bp and assigned an ''r'' number. The software for spa type, Ridom StaphType is used to classify and determine the spa type of CA-MRSA isolates[92]. *Spa* typing is rapid, high throughput with a portable dataset that simplifies information[76]. However, sequencing errors are possible with spa typing and it can misclassify particular types due to recombination and/or homoplasy[77].

Microarrays: Microarrays are widely used to identify single nucleotide polymorphisms (SNPs). There are several microarrays depending on the number and types of DNA spots on the array, the solid support, labeling system, controls for the spots, hybridization conditions, detection system, and data analysis methods. Microarrays (Sam-62) based on 62 *S. aureus* whole genome sequencing (WGS) projects and 153 plasmid sequences shows that MRSA transmission events that are unrecognized by other approaches can be identified and is capable of distinguishing extremely similar but non-identical sequences[87.] Microarray data are reproducible, take 1–2 days and highly accurate. But it is technically demanding, particularly to interpret the data and they do not allow the identification of sequences which are not included in the array[77].

Whole genome sequencing (WGS): The cost reduction in bench top sequencers and new data analysis softwares makes bacterial WGS possible even in small research and clinical laboratories and it is likely to replace currently used typing methodologies for epidemiological investigations due to its ultimate resolution[87]. WGS of MRSA isolates in an outbreak in neonatal intensive care unit (NICU), Cambridge University Hospitals showed distinct cluster of outbreak isolates (ST22) with a clear separation between these and the non-outbreak isolates based on SNP analysis [93]. Comparison of WGS of MW2 (USA400) strain and the HA-MRSA strains, Mu50 and N315 revealed that MW2 and the HA-MRSA strains are relatively distant. MW2 strain carry SCC*mec*IVa element that encodes methicillin resistance gene and penicillinase gene while N315 and Mu30 carries SCC*mec*II element that had multiple antibiotic resistant genes[94]. WGS has the potential to compare different genomes with a single-nucleotide resolution. This would allow an accurate characterization of transmission events and outbreaks. However, translating this potential into routine practice will involve extensive investigations[87].

Control measures: When CA-MRSA strains are detected, it is required to do contact tracing to identify and decolonize MRSA carriers among family and close social contacts to limit the community spread of MRSA strains. Screening for MRSA carriage is generally performed by swabbing the anterior nares which has a sensitivity of over 80% that can be increased to 95% when additional sites such as throat, sputum, perineum, and rectum are screened. Decolonization therapy with mupirocin combined with chlorhexidine body washes and oral rifampicin and doxycycline for 5 to 14 days (median 7 days) gives successful MRSA eradication[66].

CONCLUSION

Rapid methods for MRSA identification and typing are essential to determine the transmission in the community. Molecular typing methods such as SCC*mec* typing, *spa* typing, MLST and PFGE are recognized as promising methods for MRSA surveillance because of their capability to characterize bacterial chromosomes and mobile genetic elements. Combination of these molecular typing methods is necessary as each method has its own advantages with respect to discriminatory power, rapidity, cost effectiveness, reproducibility, and easy performance.

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