MRSA: The Resistance Is Getting More Difficult

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***World MRSA Day on October 2, 2010**

Methicillin-resistant *Staphylococcus aureus* (MRSA) has long known as an important multidrug resistant bacterial pathogen that plays a threatening role amongst nosocomial infection or patients who have stayed in long-term health care facilities. Infection due to MRSA causes a substantial health care burden because of a longer duration of hospital stay, a limitation of treatment options and a difficulty in managing infection complications, and thus resulting in higher morbidity and mortality rates. Prevalence of MRSA infection varies greatly according to geographic regions from less than 5% in several European countries to over 50% in many Asian countries. During the last decade, an increase of community-acquired infection due to MRSA has raised more attention due to its rapid spread and the severity of the disease it may cause. Not surprisingly, a rise of infection rate due to MRSA, which is resistant to all beta-lactam agents, leads to a heavy use of glycopeptides, the antimicrobial agents of choice (e.g. vancomycin). MRSA strains with decreased susceptibility to glycopeptides, as a result, have been emerged recently, and are impending for their spread unless rigorous infection control is implemented. Newer antimicrobial agents with activity against MRSA are currently available in some limited countries, but remain to be further evaluated for clinical use. This review will discuss various aspects of MRSA including background (structural and molecular characteristics), laboratory detection, susceptibilities to antimicrobial agents, prevalence including local data and control measures.

History of MRSA

More than a decade after being discovered by Alexander Fleming in 1928, benzylpenicillin had been effectively used against infections due to various bacteria including *S. aureus.* In 1950s, resistance to penicillin recognizably emerged among staphylococci and was mostly due to the production of plasmid-mediated penicillinase, a form of beta-lactamase that cleaves beta-lactam rings of the penicillin structure which its encoding gene spreads easily by a mobile genetic element. With only about a decade of use, benzylpenicillin was no longer useful against *S. aureus.* To battle penicillin-resistant *S. aureus*, methicillin, a penicillinase-resistant penicillin, was introduced in 1959. It was truly tragic that the first case of MRSA infection rendering methicillin ineffective was identified from the United Kingdom in 1961, shortly after the use of methicillin. Thereafter, MRSA strains were rapidly spread and became endemic worldwide, especially in hospital settings. Hospital-acquired MRSA isolates are also resistant to multiple classes of antibacterial agents. Until the late 1990s, the emergence of MRSA causing community-acquired infection had been reported from previously healthy patients who had no identifiable risks for contracting MRSA from health care settings.[1] Since then, MRSA is known to be pandemic without boundaries.

Molecular Biology and Virulence of MRSA

The emergence of MRSA was mediated by alterations in penicillin-binding protein (PBP), the drug's target site. A gene responsible for the production of altered PBP, known as PBP2a, is *mecA*, which is located on a genetic mobile element "Staphylococcal Cassette Chromosome (SCC) *mec*".[2] Expression of PBP2a, a 78 kDa protein involved in bacterial cell wall synthesis, results in a loss of drug target affinity, i.e. the capability of drug binding to cell wall is greatly reduced. This phenomenon affects most forms of beta-lactam agents including penicillins, cephalosporins and carbapenems. The SCC*mec* element is uncertain in its origin, but is widely disseminated among staphylococci. This genetic element can be integrated into bacterial chromosomes by site-specific recombination, and is capable of both horizontal and vertical transfers. Thus, it can be spread very quickly. There are up to eight types of SCC*mec* that have been described to date and each type has its own resistance characteristics.[3,4] Most of health care-associated (HA) MRSA isolates possess SCC*mec* types I- III which also carry other resistance determinants conferring resistance to aminoglycosides, tetracyclines, macrolides and lincosamides. In the other aspect, community-associated (CA) MRSA isolates usually carry SCC*mec* type IV which contains only the *mecA* gene and is often susceptible to non-beta-lactam antibiotics. Compared to HA-MRSA, CA-MRSA is usually susceptible to chloramphenicol and clindamycin. However, CA-MRSA may acquire resistance to other antibiotics via various mechanisms. For examples,

CA-MRSA isolates may be resistant to erythromycin, but not clindamycin, via an *mrsA* gene-mediated efflux pump. If CA-MRSA isolates carry the *erm* gene which causes ribosomal methylation, they will be resistant to erythromycin with a characteristic of inducible clindamycin resistance during clindamycin therapy. Both HA-MRSA and CA-MRSA are usually susceptible to trimethoprim/sulfamethoxazole, but are resistant to erythromycin. While HA-MRSA is usually resistant to fluoroquinolones, susceptibility of this drug class among CA-MRSA strains is various in different geographic regions.

Several studies have recently focused on the pathogenesis of CA-MRSA.[5-7] CA-MRSA emerges through a recombination of hospital-derived MRSA strains and drug-susceptible community strains of *S. aureus*. Such recombination results in strains that are naturally fit in the community and can cause disease in healthy individuals including severe infections. It has been shown in animal models that CA-MRSA strains are in fact more virulent than are HA-MRSA strains.[2,5,7] Panton-Valentine leukocidins (PVLs) or leukotoxic proteins (e.g. LukS-PVL and LukF-PVL), are believed to be the major virulence factors of CA-MRSA that are absent in HA-MRSA. PVL proteins induce chemotaxis (i.e. neutrophil infiltration), secretion of degradative enzymes and generation of free radicals resulting in an inflammatory response and eventually tissue necrosis. However, PVL-negative CA-MRSA remains to be virulent. It was later demonstrated that alpha-toxin and alpha-type phenol-soluble modulins are also essential virulence factors of CA-MRSA.[2,6] Both substances mediate lysis of several host cells, induce inflammation and have an important role in the virulence of PVL-negative CA-MRSA strains.

Clinical Significance of MRSA

Although a large human population is believed to be colonized with staphylococci on their skin and nostrils, a survey in the USA showed that only a small portion (approximately 2%) is colonized with MRSA.[8] These colonized individuals, however, may also be attacked by their own MRSA, so called endogenous infection, if the bacteria spread to other body parts where they can initiate inflammatory responses. Infections due to MRSA are generally similar in spectrum to what is caused by methicillin-susceptible *S. aureus* (MSSA), but may lead to a more invasive disease and are complicated by the difficulty of treatment. MRSA infection often involves skin and soft tissue, but other various presentations such as meningitis, pneumonia, surgical site infection, organ abscess, osteomyelitis and septicemia may also occur.[3,7,9] A survey in the USA demonstrated that most invasive MRSA infections (approximately 85%) are associated with health care.[9] Important risk factors for acquiring HA-MRSA include previous history of health care contact, long-term hospitalization, admission to intensive care unit, receiving invasive treatment procedures, implantation of medical device and exposure to antibiotic treatment. Health care personnel have a crucial role in the transmission of HA-MRSA among different patients, known as cross-infection, via personal contact during physical examination and contaminated medical equipment. The risk factor of CA-MRSA infection is not clearly defined, but patients with skin and soft tissue infection pose a greater risk for acquiring CA-MRSA. Participation in contact sports, activity with skin-to-skin contact, patients who have a cut or abrasion of skin and living in poor sanitation conditions may also increase their chance of acquiring CA-MRSA.[3,7,10] Diagnosis of MRSA infection is readily made through appropriate isolation of the organism from the infection site.

Vancomycin is recommended as the first-line therapy for invasive infection due to MRSA, particularly HA-MRSA. However, it has been demonstrated that MRSA isolates with vancomycin MIC over 1 mg/L are significantly related to clinical failure with vancomycin treatment despite a susceptibility breakpoint of 2 mg/L.[11,12] Thus, vancomycin MIC determination and monitoring of serum level of vancomycin to attain trough concentrations of 15-20 mg/L during therapy are critical for effective treatment and to avoid drug toxicity.[10] In non-invasive infections, oral preparations such as trimethoprim/sulfamethoxazole, long-acting tetracyclines (e.g. doxycycline and minocycline) and clindamycin are recommended for treatment of CA-MRSA. Rifampicin and fusidic acid may also be used as alternatives, but both agents should be used in combination with other agents because resistance emerges easily during single agent therapy. For serious cases of CA-MRSA infection, more potent drugs such as vancomycin and fluoroquinolones should be used. However, prolonged treatment of staphylococci with quinolones may induce resistance development and isolates could become resistant to quinolones after 3-4 days of therapy. Thus, repeat testing of isolates may be indicated especially if delayed response to treatment is suspected. Newer agents that have potency against MRSA, but often are preserved for serious infections, include daptomycin, linezolid, quinupristin-dalfopristin, tigecycline and ceftobiprole. Additionally, if pus collection is observed, appropriate drainage or surgical procedures are required besides antimicrobial therapy. Several epidemiological typing techniques such as pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MSLT), *spa* gene typing and SCC*mec* genotyping are widely used for molecular epidemiologic study, but are of little clinical use since they cannot predict the drug susceptibility of individual strains.[2,10] The treatment option is based directly on the susceptibility testing result of each isolate.

Detection and Susceptibility Testing of MRSA

A latex agglutination test for detection of PBP2a and nucleic amplification of *mecA* gene are probably the most accurate methods of determining MRSA, but may have not been widely used in the clinical laboratory. The Clinical and Laboratory Standards Institute (CLSI) has recommended the susceptibility testing of oxacillin or cefoxitin as a means for detection of MRSA based on the disk diffusion or broth dilution or agar dilution method.[13] According to the minimal inhibitory concentration (MIC), strains with MIC of oxacillin or cefoxitin of equal to, or over 4 or 8, respectively, should be reported as MRSA. Several studies reported that interpretation based on cefoxitin showed higher sensitivity and specificity for detection of MRSA than the use of oxacillin, and therefore cefoxitin can be used as a surrogate for the detection of MRSA.[13] Upon determination as MRSA, isolates are entitled to be resistant to all currently available beta-lactam agents including cephems,

carbapenems and beta-lactam/beta-lactamase inhibitor combinations, except only for newer cephalosporins with anti-MRSA activity (e.g. ceftobiprole). Therefore, testing for beta-lactam antibiotics other than oxacillin or cefoxitin is not recommended. If tested, however, all beta-lactam agents should be reported as resistant to these drugs regardless of in vitro results since clinical use is not suggested.

Vancomycin is considered a drug of choice for treatment of infection due to MRSA. Previously, a number of clinical laboratories tested vancomycin susceptibility against *S. aureus* by the disk diffusion method. In 2009, however, CLSI notified that testing *S. aureus* against vancomycin by the disk method cannot differentiate vancomycin-susceptible from vancomycin intermediate isolates i.e. both categories may yield similar inhibitory zone sizes.[13] Therefore, disk testing is considered unreliable for testing vancomycin against staphylococci. A disk test can only detect *vanA*-containing or vancomycin-resistant *S. aureus* (VRSA) which, in this case, isolates will have no inhibition zone. Isolates suspecting to be VRSA should be confirmed for the presence of the *vanA* gene. Reporting of vancomycin susceptibility for *S. aureus* should therefore be in terms of MIC and isolates with vancomycin MIC less than or equal to 2 mg/L are interpreted as susceptible. Isolates with MIC equal to or over 4 mg/L should be confirmed by a reference laboratory. VRSA isolates are determined if vancomycin MIC is equal to or over 16 mg/L. It should be noted that reliability of susceptibility testing of teicoplanin by the disk diffusion method has not been reevaluated whether the similar phenomenon will appear as in vancomycin. Therefore, disk diffusion breakpoints for teicoplanin remain available, but the accuracy for determining intermediate and resistant strains is unknown.[13]

It is also recommended that all staphylococcal isolates, except those from the urinary tract, with the original susceptibility results of erythromycin-resistant and clindamycin-susceptible should be further determined for inducible clindamycin resistance due to *erm* gene-mediated ribosomal methylation by a D-zone test or broth microdilution. The D-zone test is more practical for use in a clinical laboratory as it is based on the disk diffusion method. Briefly, erythromycin and clindamycin disks are placed on top of isolateoverlaid Mueller-Hinton agar with 15-26 mm apart. After 16-18 hours of incubation at 35 degree Celsius, flattening of the clindamycin inhibition zone only at the side that is adjacent to the erythromycin disk, as seen in D-shape, indicates inducible clinidamycin resistance (Fig 1). If this is the case, the isolate should be reported as clindamycin-resistant even though it was originally tested as susceptible.

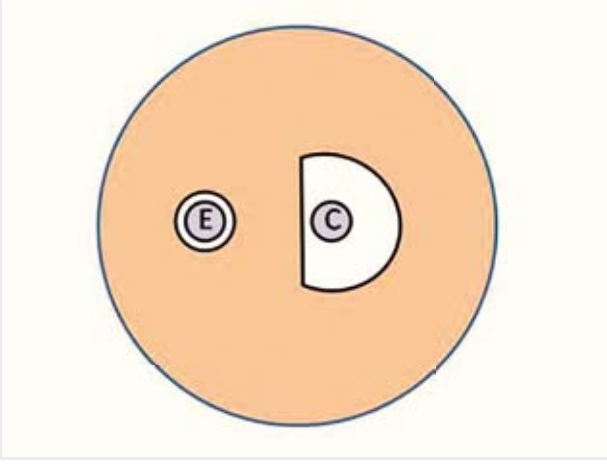

Fig 1. D-zone test. A D-shape appearance of clindamycin inhibitory zone with flattening zone adjacent to erythromycin disk indicates inducible clindamycin resistance. (E, erythromycin; C, clindamycin)

CLSI also provides method and interpretation guidelines for some newer antimicrobial agents with anti-MRSA activity such as linezolid, quinupristin-dalfopristin and daptomycin.[13] For daptomycin, the disk diffusion method is still considered not reliable and MIC testing is required. The isolate with non-susceptibility to linezolid and daptomycin has not been well documented and thus resistant breakpoints are not provided. Isolates suspected to be non-susceptible to aforementioned agents should be referred to a reference laboratory to further confirm their susceptibility. Other newer drugs such as tigecycline and ceftobiprole have up to now no susceptibility testing and interpretation guidelines.

Local data on MRSA

The true prevalence of clinically significant MRSA amongst *S. aureus* isolates in Thailand has never been thoroughly evaluated. A limited survey at 28 public Hospitals by the National Antimicrobial Resistance Surveillance, Thailand during the year 2000-2005 suggested that rates of MRSA were approximately 24%-27%.[14] At Siriraj Hospital, a large tertiary-care university hospital in Bangkok, the prevalence of MRSA among *S. aureus* isolates during 2007, 2008 and 2009 were 46.5%, 41.0% and 48.3%, respectively. The antibiogram of *S. aureus* clinical isolates (MRSA and MSSA) at Siriraj Hospital for various antimicrobial agents during 2007-2009 is shown in Table 1. A random survey of MRSA isolates in 2008 (n = 100, 57% from respiratory tract) revealed that the MIC range of vancomycin was 0.75-2 mg/L with MIC_{50} 1.5 mg/L and MIC_{90} 2 mg/L. In 2009, a survey of 564 *S. aureus* blood isolates (MRSA 38.1%, MSSA 61.9%) demonstrated vancomycin MIC ranges of MRSA and MSSA to be 0.5-3 mg/L (MIC_{50} 1.5 mg/L and MIC_{90} 2 mg/L) and 0.75-3 mg/L

TABLE 1. Antibiogram of *S. aureus* during 2007-2009 at Siriraj Hospital.

Organism	Year	Number	% Susceptible isolate								
			Vancomycin	Gentamicin	Ciprofloxacin	Chloramphenicol	Erythromycin	Clindamycin	Tetracycline	Co-trimoxazole	Linezolid
MRSA	2007	1340	100	11	0	92	1	0	9	12	100
	2008	955	100	12	0	95	1	1	10	22	100
	2009	1283	100	8	1	94	1	1	22	50	100
MSSA	2007	1542	100	98	88	95	91	92	64	98	100
	2008	1423	100	98	90	94	88	88	64	99	100
	2009	1479	100	99	89	95	89	90	60	99	100

(MIC_{50} 2 mg/L and MIC_{90} 2 mg/L), respectively. Interestingly, 75% of MRSA isolates had vancomycin MIC at least 1.5 mg/L and 33% of isolates had vancomycin MIC of 2 mg/L. In addition, 70% of MSSA isolates had vancomycin MIC at least 1.5 mg/L and 48% of isolates had vancomycin MIC of 2 mg/L. Given that blood isolates of *S. aureus* are very likely to be clinically significant and the vancomycin MIC breakpoint is 2 mg/L, it is of serious concern that one isolate of each MRSA and MSSA isolated from blood in this survey showed vancomycin MIC of 3 mg/L that is considered to be vancomycin-intermediate. From this survey, three important points should be noted: 1) a large number of *S. aureus* isolates, both MRSA and MSSA, demonstrated vancomycin MIC values in an upper range of susceptible MIC or close to be called non-susceptible, 2) MSSA isolates did not have any significant difference in vancomycin MIC as compared to MRSA isolates, and 3) clinical isolates of vancomycin-intermediate *S. aureus* (VISA) have emerged at this hospital. Moreover, a majority of isolates had vancomycin MIC over 1 mg/L which has demonstrated that use of vancomycin in these patients could result in clinical failure.[11,12] Therefore, close monitoring of vancomycin MIC, measuring of vancomycin serum level and clinical judgment of antibiotic use are crucial for effective management and control of MRSA infection at this hospital.

Epidemiology and control of MRSA

Some strains of MRSA are capable of being widespread in particular regions which may be annotated as epidemic MRSA or EMRSA. For example, EMRSA-15 and EMSRSA-16 emerged and spread among several hospitals in the United Kingdom during 1990s. In the USA, CA-MRSA pulsed-field type USA400 or sequence type (ST) 1 was most predominant before 2001.[15] Since then, clone USA300 or ST8 has emerged and became the leading cause of community-acquired infection due to MRSA.[16] Clone USA300 is not genetically related to USA400 and has not been found to be associated with hospital-acquired MRSA infection. Among HA-MRSA, clones USA100 and USA200 are more common.[3] The most important mean for the spread of MRSA is direct skin-to-skin contact. Poor hygiene and a crowded community are also risk factors to facilitate the dissemination of the organism. Therefore, hand washing with antiseptic soaps (e.g. 0.3% triclosan) or alcohol-based hand rubs (e.g. 62.5% ethyl alcohol) is considered the most effective measure to prevent cross-transmission or spread of MRSA. Skin disinfectants such as chlorhexidine and tincture iodine are also efficient to eliminate MRSA. Proper personal hygiene, i.e. sharing of personal items is not recommended, and avoid contacting with wounds are also important to prevent MRSA infection especially among hospitalized patients, students, athletes and inmates who are at greater risk for personal contact. Health care personnel are universally required to be aware of appropriate hand hygiene prior to performance of physical examination to or make contact with patients. Screening and eradication of nasal carriage of health care staff and patients may be less efficient due to a possibility of extranasal carriage. The effectiveness of mupirocin, commonly used to eliminate nasal colonization of MRSA, is still controversial. Patients colonized or infected with MRSA may be arranged in separate areas with restricted access. Health care personnel and visitors should wear gloves and gowns prior to having physical contact with MRSA infected patients, and discard these personal protections and wash their hands appropriately before leaving the patient's area. MRSA can also survive in the environment or inanimate surfaces for a long time. Therefore, cleaning MRSA infected patient's areas, personal belongings and equipment with disinfectant is also mandatory.

CONCLUSION

S. aureus has been a leading human pathogen for a long time. Although several antimicrobial agents have activity against this bacterium, its abilities to continuingly develop more resistant strategies and to evade host defense mechanisms allow this organism to successfully survive in nature and cause diseases in humans. This is clearly exemplified by the pandemic of MRSA infection soon after the introduction of methicillin in clinical use. Probably related to the rule of natural selection, HA-MRSA strains that are endemic in health care settings are resistant to a wide panel of antimicrobial agents, including non-beta-lactams. The emergence of CA-MRSA truly declares the great achievement of this menacing bacterium. CA-MRSA, despite a lower spectrum of antibiotic resistance, has become more vilurent than has HA-MRSA and has rapidly spread in the community to cause infection in healthy individuals. Although risk factors for acquiring CA-MRSA remain uncertain, crowded communities and physical contact activities are likely to place people at risk. Accurate detection of MRSA isolates, effective coordination of health care staff regarding control measures, prompt isolation and proper care of infected patients, appropriate antimicrobial use and continuing epidemiologic surveillance of resistance patterns are all crucial for preventing the spread of MRSA.

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