

# Methicillin-Resistant *Staphylococcus aureus* (MRSA) : Review of Problems and Comments

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## Abstract

MRSA is a major nosocomial pathogen causing severe morbidity and mortality in many hospitals worldwide. Once MRSA is established in the hospitals, it is difficult to eradicate because it is uniformly resistant to many antimicrobial agents. Like the more sensitive strains of *S. aureus*, it may be carried asymptotically (particularly in the nose) or it may cause infections of varying severity. MRSA can quickly become a significant and expensive hospital epidemiological problem if not recognized and treated. Some important aspects of MRSA are described as follows :-

- (i) Mechanisms of penicillin and methicillin resistance
- (ii) Infection control
- (iii) Bacteriological methods and identification of MRSA
- (iv) Strategies for typing of MRSA outbreaks
- (v) Treatment of MRSA
- (vi) Antimicrobial susceptibility of MRSA in Thailand

(*J Infect Dis Antimicrob Agents* 1994;11:141-7.)

**Key words** : *Staphylococcus aureus*, MRSA, methicillin-resistance

## เรื่องย่อ

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MRSA เป็นสาเหตุสำคัญของโรคติดเชื้อที่เกิดขึ้นในโรงพยาบาลหลายแห่งทั่วโลก เมื่อมี MRSA เกิดขึ้นแล้ว การกำจัดเชื้อให้หมดไปทำได้ยาก เนื่องจากเชื้อมีคุณสมบัติคือยาด้านจุลชีพหลายชนิด MRSA มีคุณสมบัติเหมือนกับเชื้อที่มีความไวต่อยาเมธิซิลลิน กล่าวคือ พบได้ในผู้ที่เป็นพาหะโดยเฉพาะในรูจมูกและทำให้เกิดโรคที่มีความรุนแรงได้หลายแบบ ปัญหาของ MRSA ในโรงพยาบาลเน้นไปทางด้านการสิ้นเปลืองค่าใช้จ่ายเพื่อการรักษาผู้ป่วย และด้านระบาดวิทยา ถ้าหากไม่ได้ทำการตรวจพบและรักษาผู้ป่วย MRSA ให้ถูกต้องได้กล่าวถึงลักษณะที่สำคัญบางประการของเชื่อดังต่อไปนี้

- (i) กลไกการดื้อยาเพนิซิลลินและเมธิซิลลิน
- (ii) การควบคุมโรคติดเชื้อ MRSA
- (iii) วิธีตรวจหา MRSA
- (iv) วิธี typing เมื่อเกิดการระบาดของ MRSA

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(v) การรักษาผู้ป่วย  
 (vi) ความไวต่อยาต้านจุลชีพของเชื้อ MRSA ในประเทศไทย  
 (วารสารโรคติดเชื้อและยาด้านจุลชีพ 2537;11:141-7.)

Staphylococci appear as gram-positive cocci, cluster-forming bacteria because of random cell division in three planes. The resultant daughter cells remain adherent together via carbohydrate surface slime that forms intercellular bridges which link the cocci together in clusters. Cocci appearing in smaller grouping of pairs and short chains result from the rupture of these intercellular bridges. The latter forms may also be visualized on a direct smear of pus from staphylococcal lesions (1).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing severe morbidity and mortality in many hospitals worldwide (2-4). Once MRSA is established in the hospitals, it is difficult to eradicate (5-6). One factor is that MRSA is not only uniformly resistant to methicillin, oxacillin, cloxacillin, nafcillin and other penicillins, it is also unfortunately resistant to many other antibiotics e.g. aminoglycosides, chloramphenicol, cephalosporin, erythromycin and tetracycline. Outbreaks of MRSA have been reported worldwide (7-8). Patients at highest risk for MRSA infections include those at tertiary care university hospitals, large community teaching hospitals, elderly patients with burns, seriously ill patients who are usually in intensive care unit, and particularly post-operative patients (2-3).

The emergence and persistence of MRSA is a major health care problem in terms of cost in the health care system. Vancomycin which is the drug of choice for treatment is very expensive (9). Patients acquiring MRSA in the intensive care unit had a longer mean stay of 10 days in a hospital compared with those acquiring methicillin-sensitive *Staphylococcus aureus* (10).

Like the more sensitive strains of *S. aureus* it may be carried asymptotically (particularly in the nose) or it may cause infections of varying severity.

#### I. Mechanisms of penicillin and methicillin resistance.

For penicillin resistance, *S. aureus* destroys the antibiotic by producing a group of enzymes called penicillinases ( $\beta$ -lactamase) so named because they hydrolyze the  $\beta$ -lactamthiazolidine nucleus of a variety of

penicillins. The penicillinases can, with greater difficulty, also hydrolyze the amide bond in 7-aminocephalosporanic acid derivatives (cephalosporin antibiotics). The production of penicillinases is controlled in *S. aureus* by an extra-chromosomal piece of DNA which is usually called a plasmid or episome. There are usually two or three copies of the penicillinase plasmids in each bacterial cell. The penicillinase plasmid is rather large in size and has a M.W. of 20 million daltons. *S. aureus* containing penicillinase plasmids existed long before the commercial advent of penicillins in the 1950's. The increase in numbers of resistant *S. aureus* reflects the selection of strains already possessing penicillinase plasmids. In addition, the capability of gene transfer from cell to cell by bacteriophages (the phenomenon known as transduction) also facilitates the spread of penicillinases among *S. aureus* (1).

The problem of penicillin resistance resulting from penicillinase production appeared to have been solved by the advent of methicillin. This first semisynthetic penicillin, which is a penicillinase resistant antibiotic, was introduced into clinical use in England in 1959. Within 2 years, naturally occurring strains of *S. aureus* resistant to methicillin were isolated (11).

The mechanism of methicillin resistance is different from penicillin resistance. Methicillin resistance is due to production of an altered penicillin-binding protein (PBP2a or PBP2' instead of PBP2). *S. aureus* produces 5 PBPs which are enzymes that catalyze the cross-linking reactions between peptidoglycan polymers, one of the final steps in bacterial cell wall assembly (12). Of all 5 PBPs, only PBP2 and PBP3 are essential for cell growth and survival, and others are not. The PBP2a has a very low affinity to bind with methicillin. Therefore, the PBP2a is available to function in cell wall synthesis (13-16) in the presence of methicillin. This form of resistance may result from mutation in bacterial nuclear DNA.

The term "methicillin-resistant" (MR) is based on historical, not physiological factors. Because methicillin became available prior to the other members of this group of drugs, and methicillin resistance was noted and

identified early, it was convenient to refer to all such resistant isolates as “methicillin-resistant” strains. Thus MRSA is also resistant to oxacillin, cloxacillin, dicloxacillin and nafcillin.

## II. Infection control.

### A. Isolation and identification of MRSA patients.

As MRSA poses a risk to other patients, patients who are either colonized or infected should be nursed in standard isolation on a special ward. Patients' notes should be tagged with a red label stating that they are or have been MRSA carriers (17).

### B. Discharge or transfer of MRSA patients.

It is advisable to discharge infected or colonized patients to home as soon as medically feasible and to advise them of the nature of their infection. Colonized patients should be discharged with topical bacitracin/mupirocin/triclosan as appropriate (17). When a patient is to be transferred to another hospital, it should be notified in advance that the patient is or has been affected by MRSA.

### C. Readmission of a previously affected patient or transfer of a patient from a hospital with an MRSA problem.

It is recommended that these patients should be admitted directly into standard isolation in a sideroom, where screening for MRSA carriage should take place. The standard isolation should not be discontinued until clearance screens have been obtained. If the patient was known to be still carrying the MRSA immediately prior to readmission, there should be a special ward where strict control measures could be implemented (17).

### D. Screening swabs.

The following swabs should be taken (17) : nose (both nostrils on one swab), hands (both on one swab), axillae (both on one swab), perineum, wounds, abnormal skin (e.g. eczema, psoriasis), and any known previously positive site.

### E. Swab method.

The swab should be moistened in sterile normal saline solution or peptone water and then rubbed vigorously over the site approximately 5-10 times. The swabs should then be sent to the Bacteriology Laboratory immediately (17).

### F. MRSA clearance.

Swabs are not the ideal way of isolating the organism and so it is necessary to have three clear sets

of screening swabs before it can be assumed with a reasonable degree of certainty that the patient is clear of MRSA. The screening swabs are usually taken at weekly intervals (17).

### G. Several MRSA patients on one ward.

It is necessary to perform ward screening and decontamination procedures. For physicians, nurses and other medical personnel, the most important specimens to be sent to the Bacteriology Laboratory for MRSA culture are nasal swabs and hand swabs. In some studies, hand washing samples were collected using nonbacteriostatic, sterile 0.9% saline instead of hand swabs (17-18).

For environmental control, air sampling cultures are performed by using blood agar plates whose lids are opened near the MRSA patient's bedside. After 15 min, the lids are replaced and the plates are sent to the Bacteriology Laboratory for culture. The screening swabs are also taken from area around the MRSA patients e.g. drapery, bedsheet etc.

### H. Avoidance of excessive use of antibiotics in uncontrolled conditions (a prophylactic strategy).

It is believed that the excessive use of antibiotics may pose a selective pressure for the existing and spreading of MRSA.

## III. Bacteriological methods and identification of MRSA.

All specimens are inoculated on mannitol salt agar or blood agar, incubated at 35°C and examined for growth at 24 and 48 hours. Gram-positive, catalase-positive cocci are identified as *S. aureus* when colonies yield a positive result with the slide coagulase or tube coagulase test (19-20).

To determine whether *S. aureus* is MRSA or not can be done by several methods. The easiest and most convenient method for practical use is the disk diffusion method.

**1. Disk diffusion method.** This method is a reliable method for detection of methicillin resistance only if some precautions are taken because MRSA has a special characteristic. The methicillin resistance is typically heterogeneous. Only rare cells (1 in  $10^4$  to  $10^8$ ) express the resistance trait and grow in the presence of high concentrations of drug. Most cells appear susceptible to relatively low, therapeutically achievable concentrations of drug. Thus, heterogeneous strains can be considered to be composed of two populations of cells : relatively

susceptible cells and highly resistant cells (4,20-24). Strains that show heterogeneous resistance when grown at 37°C may appear homogeneous resistant when grown at 30°C or if 4% NaCl is added to the medium. NaCl enhances the expression of resistance through effects on the susceptible subpopulation or cells, not the resistant subpopulation. Thus the disk diffusion method is reliable if 4% NaCl is added to the Mueller-Hinton agar.

*S. aureus* suspension which has been adjusted to an appropriate density is spreaded onto Mueller-Hinton agar + 4% NaCl by using a sterile swab to ensure an even distribution of the inoculum. An oxacillin disk (1 µg) is placed on the inoculated plate and pressed firmly into the agar with sterile forceps to ensure complete contact with the agar. The oxacillin disk is better than methicillin and nafcillin disks because it is the best standardized for the disk diffusion method, the most stable and the most reliable. The relative instability of methicillin disks in storage makes their use less desirable. After incubation at 35°C for 24 hours, the agar plate is examined for an inhibition zone around the oxacillin disk. If the zone is less than 13 mm. in diameter the strain tested is MRSA.

**2. Broth microdilution.** In this method for determining MRSA, specific amounts of methicillin prepared in decreasing concentration in Mueller-Hinton broth containing 2% NaCl by two-fold serial dilution technique are inoculated with *S. aureus* suspension (24-25). The least amount of methicillin which inhibits bacterial growth after incubation at 35°C for 24 hours is referred to as the minimal inhibitory concentration (MIC). If the MIC of *S. aureus* is more than 8 µg/ml, it is MRSA.

In the case that oxacillin is used instead of methicillin, the MIC of more than 16 µg/ml is the cut off value for MRSA.

**3. Agar screen.** In this method, *S. aureus* suspension is inoculated onto agar containing methicillin. Growth of any colonies on this drug-containing agar is indicative of resistance.

An inoculum of *S. aureus* 10<sup>4</sup> CFU (colony-forming unit) is spotted onto Mueller-Hinton agar + 4% NaCl containing methicillin 10 µg/ml or oxacillin 6 µg/ml. After incubation at 35°C for 24 hours, the agar is examined for growth of colonies. Growth of even a single colony is indicative of MRSA (4, 26).

#### IV. Strategies for typing of MRSA outbreaks.

Nosocomial transmission of MRSA within a clinical ward literally at the hands of hospital personnel has been a familiar story (27-29). For example, MRSA was isolated from 92 patients in one hospital (27). Out of these, 82 patients developed MRSA infection, mostly in the form of surgical wound infection. It was found that 3 surgical ICU nurses were nasal carriers of MRSA, and one of them had dermatitis on both hands which was colonized with MRSA. Another example was a burn patient who was transferred from one hospital to another hospital (28). Despite standard wound precautions, transmission to 34 patients occurred during the subsequent 15 months, and seventeen of the 34 patients died. Screening of the medical personnel for MRSA was conducted in all physicians and 128 nurses. It was found that one nurse had chronic dermatitis and otitis externa. MRSA was isolated from many areas e.g. ears, nose, hands, axillae and rectum.

Whenever there is an MRSA outbreak, it is important to understand the mechanism of MRSA cross-infections of other patients. To establish whether one particular strain of MRSA is involved, or whether multiple strains are causing infection, a collection of isolates needs to be characterized to follow the spread of particular strains.

**1. Antimicrobial susceptibility.** This method is not widely used because MRSA is uniformly resistant to many antimicrobial agents. Thus, if two strains of MRSA have the same resistant antibiogram, they may not be the same strain. The antibiogram will be useful only if there are some differences e.g. one strain is sensitive and the other is resistant to a particular antimicrobial agent.

**2. Phage typing.** Isolates of *S. aureus* have long been typed by their susceptibility to lysis by a series of bacteriophages, and this has been invaluable in documenting their spread (30-32).

Bacteriophages are viruses which infect bacteria. Typing is based on the phage lysis of susceptible bacterial cells, and lack of lysis of resistant ones. The patterns formed by lysis, or lack thereof to a set of standard phages permit an epidemiologic typing. The technique is as follows: *S. aureus* is spreaded evenly on a nutrient agar by using a swab. Phages are then individually inoculated on the agar e.g. 20 spots for 20 phages. After incubation at 37°C for 24 hours, zones of lysis are noted and the number of clear (lytic) plaques counted.

The phage type of a given strain is indicated by listing all of the phages which result in positive reactions i.e. zone of lysis.

**3. Molecular technique.** Many strains of MRSA are untypable by phages. The molecular approach to discrimination between MRSA strains has proved very valuable (33-34). Restriction enzyme digestion of chromosomal DNA is a good discriminatory method. The method is summarized as follows :

For DNA digestion, DNA samples are cleaved with restriction endonuclease Bgl II overnight at 37°C. The resulting fragments are separated by gel electrophoresis using 0.9% w/v agarose at 120 V.

For interpretation of results, if one particular MRSA strain is involved in infection, the DNA fragments resulting from chromosomal DNA digestion with restriction endonuclease Bgl II will yield the same pattern after gel electrophoresis. If multiple MRSA isolates are involved, the DNA fragments will be different.

## V. Treatment of MRSA.

The treatment has two aspects as follows :

**A. Treatment of colonization with mupirocin cream/ ointment for localized carriage and antiseptic bathing (e.g. triclosan)/shampooing for more widespread carriage. The antiseptic solution should be stopped if the skin is adversely affected by it (17).**

It has been reported that mupirocin nasal ointment applied to the anterior nares harboring MRSA four times daily for two days usually eliminates MRSA within 24-48 hours. Ward personnel who are nasal carriers of MRSA can, provided that other carriage sites remain negative, resume normal work after two days of this regimen (35-37).

Some other regimens have been used to eliminate MRSA in asymptomatic carriers. An example is rifampicin (300 mg twice daily for 5 days) with or without trimethoprim-sulfamethoxazole (38). Ciprofloxacin has also been used as an alternative in many institutions. However, recent reports showed that high-level resistance (MIC 90, 64 µg/ml) developed in MRSA and increased at an alarming rate, from none to 79% over a 1-year period of study (39). Therefore, ciprofloxacin appears to have limited usefulness in treating MRSA colonization and also for MRSA infections.

These results confirm that the majority of MSSA isolates carry a penicillinase factor. They also show the

widespread resistance of MRSA (and to a lesser extent MSSA) to a large variety of non-related antibiotics. This property may be due to the presence of plasmids carrying multiple resistance factors in MRSA cells.

**B. Treatment of infection.** Vancomycin is the drug of choice for treatment of MRSA infections and also for coagulase-negative staphylococci such as *S. epidermidis* (4). Vancomycin has proven efficacy against MRSA for treatment of serious infections, such as endocarditis, even when other therapies have failed (17, 40-41). It is an expensive and potentially toxic antibiotic for patients. Therefore, the level of this antibiotic in patients must be assayed regularly. Resistance to vancomycin *in vitro* has not been reported for MRSA. Vancomycin inhibits ribonucleic acid (action on DNA-dependent RNA polymerase enzyme) and cell wall synthesis and has lethal membrane effects (4, 42-43). Activity at three sites may account for the lack of development of resistance to vancomycin.

Other drugs which are also effective in treatment of MRSA infections include fusidic acid and fosfomycin. Co-trimoxazole (trimethoprim-sulfamethoxazole) should be used in non-serious infections. Rifampin and quinolones (such as ciprofloxacin) should be used as a combined therapy, not as a single drug, because resistance develops quickly (44).

## VI. Antimicrobial susceptibility of MRSA in Thailand.

The MRSA strains isolated from clinical specimens from different patients admitted to Siriraj Hospital, Bangkok (the largest hospital in Thailand) during 1988 to 1992 were studied by a modified Kirby-Bauer method designed for MRSA. The results revealed that all strains were sensitive to vancomycin (N = 94), fusidic acid (N = 94), fosfomycin (N = 31) and ofloxacin (N = 66). The sensitivity of MRSA to clindamycin (N = 64), trimethoprim-sulfamethoxazole (N = 94), ciprofloxacin (N = 64) and imipenem (N = 31) were 97%, 68%, 92% and 13% respectively. All strains were resistant to ampicillin/sulbactam and amoxicillin/clavulanic acid (45).

In more recent results, the MRSA strains (N = 30) isolated from clinical specimens from different patients admitted to Siriraj Hospital during a 3 month period starting from June 1993 were studied. The result is shown in Table 1 (46).

**Table 1. Antimicrobial susceptibility test of MRSA.\***

Antimicrobial agent	% susceptible
Penicillin G	0
Ampicillin	0
Amoxicillin/clavulanic acid	30
Methicillin (Staphycillin)	0
Cloxacillin (Orbenin)	0
Imipenem (Tienam)	44
Ampicillin/sulbactam	15
Erythromycin	22
Chloramphenicol	48
Co-trimoxazole (Bactrim)	56
Vancomycin (Vancocin)	100
Ciprofloxacin (Ciprobay)	19
Kanamycin	7
Gentamicin	26
Amikacin (Amikin)	30
Netilmicin (Netromycin)	56
Cefamandol (Mandol)	22
Ceftriaxone (Rocephin)	37

\* MRSA = methicillin-resistant *Staphylococcus aureus*.

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