Laboratory Diagnosis of CA-MRSA

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Background
The definition of CA-MRSA infection is continuing to undergo evolution. Since its initial recognition based on epidemiological criteria, hence the term "community-associated", information on various aspects of this infection is being accumulated. Clinically, it has been recognised that CA-MRSA strains have a propensity to cause skin and soft tissue infections of considerable severity, and in some cases necrotising pneumonia. Laboratory characterisation has also revealed specific features exhibited by these strains. As for epidemiological associations, infections found to fulfil clinical and laboratory criteria are increasingly documented within hospital settings.1,2

Bacteriology
S. aureus is a Gram positive bacterium commonly encountered in the clinical setting, either as an agent of infection or colonisation. Clinical specimens from which the detection of S. aureus may indicate active infection include superficial or deep wound swabs, respiratory specimens, blood cultures, etc. For the detection of colonisation, nasal, throat, axilla and other superficial swabs are cultured. Regarding susceptibility to antimicrobials, currently, most S. aureus isolates are resistant to penicillin, mediated by the production of β-lactamase. As for resistance to penicillinase-stable penicillins, such as cloxacillin, strains harbouring such resistance are termed MRSA and are mainly found in hospital settings until 1990’s. The mechanism for methicillin resistance in S. aureus is mainly due to the presence of the mecA gene, encoding an altered penicillin-binding protein with decreased affinity to various penicillinase-stable penicillins. Less frequently, resistance to methicillin may be due to hyperproduction of β-lactamase or methicillinases.3

Since the first recognition in the 1990’s of community-acquired MRSA infections typically with skin and soft tissue abscesses and necrotising pneumonia, various studies have been performed to characterise the infecting strains. In 2002, whole genome sequencing data of a CA-MRSA strain, MW2, was first reported.4 It was associated with fatal septicemia and septic arthritis in a 16-month-old American-Indian girl in 1998 in North Dakota, USA. Subsequently, a worldwide collaborative study has shown that CA-MRSA strains share two unique molecular features: harbouring staphylococcal cassette chromosome mec (SCCmec) type IV and positive for the Panton-Valentine leucocidin (PVL) gene.5 SCCmec is a genetic element containing the mecA gene. Strains carrying types I to III SCCmec are mainly hospital-related. Conversely, type IV strains in its simple truncated form are postulated to confer survival advantage to the strain in the community setting, where antimicrobial selective pressure is much lower than in the hospital setting. More recently, type V SCCmec was described to be harbour by S. aureus strains which behaved similarly to type IV strains, causing typical CA-MRSA infections.6 Regarding PVL, this is a bacterial toxin acting on leucocytes, and has been found to be associated with recurrent, often severe primary skin infections and necrotising pneumonia.7

Laboratory recognition
Laboratory detection of S. aureus in clinical specimens plays a role in the definitive diagnosis of the aetiology of an infection, and provides the opportunity for determination of antimicrobial susceptibility of the isolate, guiding appropriate therapy and contributing to baseline epidemiological information. In the clinical microbiology laboratory, S. aureus is considered as a potential pathogen when isolated from any specimen, and identification and susceptibility results will be
reported. Laboratory identification of *S. aureus* isolates is relatively simple, relying mainly on macroscopic and microscopic morphological findings, together with a positive coagulase test.

Antimicrobial susceptibility testing is mainly undertaken in laboratories in Hong Kong based on the method recommended by the Clinical and Laboratory Standards Institute (CLSI) of the United States. A positive β-lactamase test will indicate resistance to penicillin, while resistance to other antimicrobials, including methicillin, is usually tested using the disk diffusion test. The presence of methicillin resistance is indicated by resistance to the agent cefoxitin as a surrogate. Cefoxitin resistance has been shown to be highly sensitive and specific for the presence of the *mecA* gene in staphylococci. Methicillin resistance that is mediated by β-lactamase hyperproduction will be detectable by a positive β-lactamase test together with the disk diffusion test for oxacillin demonstrating resistance, while the isolate will be shown to be cefoxitin susceptible.

**CA-MRSA diagnosis**

On isolating any MRSA strains with clinical and epidemiological suspicion of CA-MRSA, further laboratory characterisation needs to be undertaken to support the diagnosis. SCC*mec* typing is performed by determining the combination of two attributes: the class of the *mec* gene complex, and with the type of the *ccr* (chromosomal cassette recombinase) gene complex. The former comprises classes A to C, and the latter comprises types 1 to 3. The technique employed is polymerase chain reaction (PCR), either using individual reactions or in a multiplex format. In addition, the presence of the PVL gene is also detected by PCR. The turnaround time of these molecular characterisation tests is one day. Currently in Hong Kong, MRSA strains harbouring SCC*mec* type IV or V, together with the presence of the PVL gene, are designated CA-MRSA. Although CA-MRSA strains are generally considered to be susceptible to most non-β-lactam antibiotics (Figure 1), multi-resistant phenotypes are not uncommonly encountered, such that the presumptive designation of non-multi-resistant MRSA strains as CA-MRSA is not reliable.

![Figure 1. Community-associated methicillin-resistant *Staphylococcus aureus* strain (note golden colour) showing resistance to β-lactams and susceptibility to multiple antimicrobials. Key: Resistant to penicillin (P) and cefoxitin (FOX); susceptible to erythromycin (E), clindamycin (DA), gentamicin (CN), tetracycline (TE), chloramphenicol (C), ciprofloxacin (CIP), rifampicin (RD) and cotrimoxazole (SXT).](image)

**Epidemiological typing**

Further typing of CA-MRSA strains is possible using various methods, including pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and *S. aureus* protein A (*spa*) typing. These methods are employed when it is necessary to delineate epidemiological relationships among CA-MRSA strains isolated from different sources, such as in outbreak settings. Typing data can also provide information on the evolution and spread of strains in a locality. PFGE can produce typing information that is comparable among laboratories only if exactly the same protocol is used. As for MLST and *spa* typing, the data obtained are objective and amenable to inter-laboratory comparison, since the information is DNA sequence-based. Between these two latter methods, *spa* typing is more discriminative than MLST. In our experience, the most common SCC*mec* type IV CA-MRSA strains in Hong Kong are of MLST sequence type 30 (the Southwest Pacific clone), constituting over a third of all CA-MRSA strains detected. The majority of these strains are of *spa* type t019. Such strains have also been designated as HKU100. Regarding SCC*mec* type V strains, the most prevalent MLST sequence type is 59, and most are of *spa* type t437.

**Screening for carriers**

One important aspect in the control of CA-MRSA is the screening of close contacts of patients for carriage of the strain. In Hong Kong, nasal and axilla swabs are obtained. In the laboratory, these are inoculated onto selective medium containing antimicrobials to suppress the growth of competing organisms. Any suspected MRSA isolates will be subjected to identification, susceptibility testing and molecular characterisation tests.

**Epilogue**

For the patient mentioned in the beginning of this article, the MRSA strain isolated was resistant only to penicillin and penicillinase-stable penicillins, and was susceptible to other classes of antimicrobials, including erythromycin, clindamycin, co-trimoxazole, tetracycline, gentamicin, otloxacin, chloramphenicol and vancomycin. Molecular characterisation revealed that the strain harboured SCC*mec* type IV, and was positive for the PVL gene. Typing of the strain showed that it was of MLST sequence type 30, and *spa* type t019. Nasal and axilla swabs from close contacts were obtained for screening, and one asymptomatic family member was found to harbour MRSA, which was subsequently characterised to have the same antibiogram and molecular characteristics as the index patient. Intranasal mupirocin and hibitane baths were prescribed, and subsequent repeat screening after the decolonisation regimen showed that the carriage was eliminated.

In order to achieve CA-MRSA control, the laboratory plays an important role in the diagnosis of the infection and screening for carriage of the organism. Clinicians are encouraged to send specimens for microbiological
investigations whenever S. aureus infections are suspected, so that antimicrobial susceptibility testing and molecular characterisation can be undertaken to guide therapeutic options and epidemiological investigations. Maintaining a close liaison of the clinical microbiologist with the attending clinician and the epidemiologist is paramount for the effective control of CA-MRSA.

Note: The Microbiology Laboratory of the Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health offers molecular characterisation tests free of charge for MRSA isolates suspected to be CA-MRSA. Contact information of the laboratory can be found at: http://www.chp.gov.hk/files/pdf/grp-specimenhandbook-en-2004122802.pdf.

References

MCHK CME Programme Self-assessment Questions

Please read the article entitled "Laboratory Diagnosis of CA-MRSA ” by Dr. Janice YC Lo, and complete the following self-assessment questions. Participants in the MCHK CME Programme will be awarded 1 CME credit under the Programme for returning completed answer sheets via fax (2865 0345) or by mail to the Federation Secretariat on or before 30 September 2007. Answers to questions will be provided in the next issue of The Hong Kong Medical Diary.

Questions 1-10: Please answer T (true) or F (false)

1. Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) is clinically distinctive in that it does not cause symptomatic disease.

2. CA-MRSA infection may still be suspected in patients with contact history with hospital settings.

3. CA-MRSA infections started to be recognised since 1990’s.

4. CA-MRSA infection is a notifiable disease in Hong Kong.

5. All CA-MRSA strains are only resistant to beta-lactam antibiotics and susceptible to other antimicrobials.

6. Suspected CA-MRSA infections should be treated with cloxacinil.

7. In Hong Kong, MRSA strains harbouring SCCmec type IV or V, and positive for the Panton-Valentine leucocidin gene, are designated as CA-MRSA.

8. On detecting a CA-MRSA case, screening of close contacts will be initiated.

9. In Hong Kong, decolonisation of CA-MRSA carriers is not undertaken.

10. The laboratory plays an important role in the diagnosis and control of CA-MRSA infections.