Molecular typing of MRSA may be performed for several reasons, but these generally fall into two broad categories. One is for microepidemiology, or the investigation of outbreaks and transmission events, where the interest is in the molecular epidemiology over a period of days to weeks. By contrast, typing to describe evolutionary relationships or the population structure of the organism is referred to as macroepidemiology, and here the timeframe of interest is years or decades.

Choosing a molecular typing method
In recent years, many new methods for molecular typing of MRSA have been described. The choice of method will depend mostly on the question being asked, since, depending on the molecular clock speed of the targets utilised in the typing scheme, some methods are best suited to answering questions regarding macroepidemiology while others are more suited for microepidemiologic studies. In addition the choice of typing method depends on factors such as discriminatory power, stability, reproducibility, ease of use, turn-around time, throughput, cost and portability of results. The following are descriptions of typing methods in routine use at CIDM-PH.

Pulse-field gel electrophoresis of SmaI digested genomic DNA (PFGE)
PFGE identifies variability on the genome which is due to the loss or gain of SmaI restriction sites. It was first described in the 1980s, but because of its high discriminatory power, it remains the gold standard for microepidemiologic studies. Since it samples the full genome, it has been successfully utilised for macroepidemiologic purposes also. Unfortunately it is slow, labour intensive and expensive, and there are issues with reproducibility and portability of results. While some of the newer rapid and inexpensive PCR-based methods have the potential to replace PFGE, they first need to be fully assessed in settings of high prevalence to prove they have comparable discriminatory power.

Multilocus sequence typing (MLST)
The mainstay of typing of MRSA for the macroepidemiologic purposes is multi-locus sequence typing (MLST). This technique involves the partial sequencing of seven housekeeping genes. Each distinct allele of a gene is assigned a unique number, and the combination of the seven different numbers gives the MLST type. It is widely accepted that the results of MLST typing produces results which reflect the true population structure of S. aureus, where most isolates appear to have evolved from a limited number of successful clones (figure 1). MLST typing is robust and easily comparable between laboratories. A web-based database for MLST typing is available (www.mlst.net). So far 1290 MLST types have been described for S. aureus.

Sequencing of 7 separate loci may be prohibitively expensive for large-scale use. Several real-time DNA amplification techniques have been described which predict the MLST clonal complex of an isolate without the need for sequencing. By computer-aided identification of seven informative single nucleotide polymorphisms within the MLST loci, Huygens and colleagues were able to develop an inexpensive and rapid method for assigning MLST clonal complex using eight SYBR-green based real-time PCR reactions[1]. This has been combined with a binary method (see below) for routine MRSA typing in Queensland.

SCCmec typing
MRSA has arisen from S. aureus by the acquisition of the cassette chromosome, SCCmec, on multiple occasions. There are at least 6 different forms of the SCCmec cassette and numerous subtypes within these. Several rapid PCR-based methods have been described to assign SCCmec types and subtypes to isolates. At CIDM-PH we use a multiplex PCR/reverse line blot assay technique (see below). Within an individual MLST clone, multiple SCCmec types can sometimes be found, indicating independent evolution and acquisition of SCCmec compared with the rest of the S. aureus chromosome. SCCmec typing is therefore frequently combined with MLST to fully characterise MRSA clones for macroepidemiologic studies. In addition the variable loci that define SCCmec subtypes are useful targets in binary typing systems utilised for microepidemiologic studies (see below).

Figure 1. Population structure of S. aureus as described by eBurst analysis of MLST data.
spa sequence typing

spa typing involves the sequencing of part of the staphylococcal protein A (spa) gene, which has a variable number of tandem repeat (VNTR) structure, spa types are assigned based on variability in both the sequence and number of repeats. Like MLST, an online database is available for electronic submission and assignment of spa types [http://spaserver.ridom.de/], and, to date, over 4500 spa types have been described. spa typing has been used successfully to monitor nosocomial transmission events in some settings [2]. In our experience most nosocomial MRSA isolates belong to only 2 or 3 spa types, so it has not proven useful for microepidemiologic studies in our setting. There is usually good correlation between spa types and MLST types, though infrequent recombination events mean that there can be exceptions.

Multilocus variable number of tandem repeat analysis (MLVA)

In MLVA typing, the amplicon sizes of several different VNTR loci are measured to estimate the number of repeats at each locus, and each unique combination of repeat numbers describes an MLVA type. There are many different VNTR loci present in S. aureus, and several MLVA schemes have been described which utilise different combinations of these [3]. Amplification of the VNTR loci is generally done in a single multiplex PCR reaction, and amplicon size determined either by gel electrophoresis or capillary electrophoresis. The latter has the advantage of greater accuracy, and the ability to identify each amplicon using dye-labelled PCR primers. MLVA has high discriminatory power, and is useful for microepidemiologic studies, however the relative instability of VNTR loci mean that MLVA results may not reflect the population structure and so would not be a useful technique for macroepidemiology.

Binary typing

Binary typing refers to typing schemes based on detecting the presence or absence of a combination of genetic loci by PCR. Amplicon detection can be performed by various methods including gel electrophoresis or real time PCR. At CIDM-PH we use multiplex-PCR followed by reverse line blot assay (RLB) for binary typing which has the advantage of high sensitivity and specificity, low cost and rapid turnaround time (less than 24 hours).

It is also a high throughput technique with up to 43 targets being detected in 43 isolates on each reusable RLB membrane, and multiple membranes may be processed simultaneously by one user [4]. To date, we have developed assays which identify 79 different binary targets in MRSA, including toxin genes, antibiotic resistance genes, SCCmec loci and phage-derived open reading frames (figure 2). Many of these targets show a high degree of variability between isolates, and we are working on developing a highly discriminatory typing system based on detection of a subset of these targets which would be useful for high-throughput microepidemiologic studies.

Conclusion

The choice of MRSA typing technique will depend on the question being asked. MLST combined with SCCmec typing is most widely used for describing macroepidemiology. PFGE remains the gold standard for microepidemiologic studies, but with further validation in high prevalence settings, new rapid PCR based methods may eventually replace PFGE for this purpose. If successful, this will allow almost real-time surveillance of MRSA isolates from hospital patients. This will rapidly identify transmission events, detect the introduction of new strains into hospitals and we hope, assist with efforts to decrease nosocomial MRSA transmission and infection.

Figure 2. Reverse Line Blot assay result for binary typing of phage-derived open reading frames in MRSA.

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Merry Christmas &
Happy 2009!

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Having an MD and postgraduate degree (PhD) in microbiology from overseas, Shahin started working at the ICPMR as a Visiting Medical Officer in May 2000. She was later appointed Research Assistant to work on a ‘Ureaplasma’ research project. In 2002 Shahin trained in Ureaplasma culture and molecular diagnosis, and started her master’s project on “Ureaplasma colonisation in women of child bearing age”. During this period she gained extensive experience in culture and molecular techniques for ureaplasma/Mycoplasma identification and serotyping.

After graduating in 2005, Shahin started working in the Pneumococcal Reference Laboratory as a Research Assistant, acquiring skills and expertise on culture and identification of pneumococcus, serotyping by Quellung reaction, serotyping by mPCR/RLB, evaluation of susceptibility testing methods for pneumococci, and reporting results to and liaising with NSW Health Communicable Diseases Branch.

Following her successful post as Research Assistant, Shahin was appointed Scientific Officer, and now manages the “Pneumococcal Reference Laboratory”. In conjunction with the surveillance project on Invasive Pneumococcal Diseases (IPD), Shahin is managing a variety of research projects on IPD both internally and externally, which are mainly related to the pneumococcal vaccination program and surveillance of changes in S. pneumoniae serotype and antibiotics susceptibility trends before and after vaccination.

CIDM Public Health – Education Program

International Molecular Diagnostics for Infectious Diseases
30 April-2 May 2009, Sydney

Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital and The University of Sydney invite clinicians, scientists, laboratory managers and public health practitioners to their annual workshop “Molecular Diagnostics for Infectious Diseases”. Program includes lectures and lab demonstrations and covers:

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- Molecular diagnostics for infectious disease management and surveillance
- Laboratory management in the era of genomic microbiology

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