Chapter 23
Staphylococci

Jodi A. Lindsay

23.1 Introduction

The staphylococci are Gram-positive cocci that normally live on the skin and mucous membranes of mammals and birds. There are over 30 species described, but the major pathogen is *Staphylococcus aureus*. There is rarely reason to type the other species of staphylococci, so this chapter will focus primarily on *S. aureus* and briefly discuss other species at the end.

S. aureus is carried in the nares of the nose in about a quarter of the healthy human population. It can also be found in the throat, axillae, groin, and intestinal tract. *S. aureus* can also survive on skin for short periods and can survive desiccation on inanimate surfaces for months. It does not normally penetrate skin on its own, but if delivered into a breach of the skin such as wound or catheter site, it is capable of causing minor through to fatal infections. Patients who are already immunocompromised, elderly, and diabetic are at higher risk of infection. *S. aureus* can seed to other tissues and therefore cause bacteraemia, pneumonia, abscess, arthritis, osteomyelitis, endocarditis, meningitis, conjunctivitis, etc. In hospitals, antibiotic-resistant strains, notably MRSA, are more prevalent. New strains of MRSA are increasingly found in the community (CA-MRSA) that cause invasive skin infection in young and healthy populations [1].

23.2 Why Type?

S. aureus strains are typed for two main reasons. Firstly, at the local level, to identify clones that have unique pathogenic or epidemiological characteristics. Identification

J.A. Lindsay (✉)
Infection and Immunity, Division of Clinical Sciences,
St George's, University of London, London, UK
e-mail: jlindsay@sgul.ac.uk

I. de Filippis and M.L. McKee (eds.), *Molecular Typing in Bacterial Infections*,
Infectious Disease, DOI 10.1007/978-1-62703-185-1_23,
© Springer Science+Business Media New York 2013

of these isolates can help to predict disease prognosis and optimise management, as well as identify epidemiological patterns and strain spread in a local environment. An example is the rapid identification of typical USA300 CA-MRSA clones that cause unique types of infection in some geographical areas and may require different patient management [2]. Local typing can also be useful for the identification of the scale and scope of a local outbreak, its likely source, and behaviours that contribute to spread, so that infection control strategies can be targeted to prevent further disease. An example may be the investigation of an outbreak of an epidemic MRSA in a hospital intensive care unit, where typing can help identify which patients were part of the outbreak, confirmation of likely sources of the outbreak, and supporting evidence that the outbreak has been stopped [3, 4].

The second reason to type is usually performed by a national or international reference laboratory, investigating large-scale evolution and spread of clonal types over large geographical areas and time. These studies are particularly useful if additional data, such as antibiotic resistance, disease, and patient details, are also collected as it can help identify shifts in key pathogenic behaviour. Examples include the emergence and spread of new epidemic MRSA clones, their spread across national borders or from unique sources such as livestock (LA-MRSA), the association of PVL toxin with necrotic pneumonia, and TSST-1 toxin with toxic shock syndrome [5–9]. This greater understanding of how *S. aureus* cause disease, evolve, and spread contributes to preventative strategies, such as screening or optimised antibiotic prescribing, and may also contribute to future diagnostics and therapeutics.

Historically, phenotypic methods were used to type *S. aureus*, most notably phage typing. Molecular methods became common from the mid- to late-1990s, particularly pulsed-field gel electrophoresis (PFGE), although these methods rely on the generation of band patterns that can be difficult to reproduce. More recently, typing methods have been dominated by sequence-based analysis, including MLST and spa typing, which are easy to compare between laboratories internationally. At the time of writing, there are new technologies and discoveries being made that are likely to have a major impact on *S. aureus* typing in the near future, including the introduction of methods that identify multiple clone-specific genes, perhaps as early as during diagnosis.

Each method has advantages and disadvantages, which will be discussed, and are briefly summarised in Table 23.1. The ideal typing method is discriminatory enough to identify isolates that are truly different, but not liable to suggesting isolates are different due to minor genome instabilities. The data generated should be accurate and reproducible and available in a format that is easily compared between remote laboratories. It should be inexpensive so that enough strains can be typed to draw useful conclusions without having to justify cost. Similarly, it should use equipment that is inexpensive or can be rented for a reasonable fee. The test should be simple to perform so as to reduce human errors and to ensure that the widest number of users have access to the method. Rapid tests are useful for investigating outbreaks, where only timely results can influence outbreak management—this depends not only on the speed of test but also on logistics of transporting specimens and reporting

Table 23.1 Simplified comparison of the advantages of each *S. aureus* typing method

	Detect lineage	Detect MGE	Simple to perform	Reproducible	Low cost	Low-cost equipment	Rapid
MLST[a]	+++	–	++	+++	+	++	+
Spa typing[a]	+++	–	++	+++	++	++	+
Microarray (whole genome)	+++	+++	–	+++	–	–	–
Mini-microarray	+++	++	++	+++	++	+	+
RM test	++	–	+++	+++	+++	+++	++
PFGE	+	+	–	+	+	+	–
Phage	+	+	++	–	+++	+++	++
SCCmec PCR	–	+	++	+++	+++	+++	++
Antibiotic resistance	–	++	+++	++	+++	+++	++
Toxin PCR	–	++	++	++	+++	+++	++

+++ an important advantage, ++ competitive, + acceptable, – disadvantage
[a]If MLST or spa typing is to be used for hundreds of strains, the cost per test can be lowered by purchasing or renting your own sequencer

results, and this is more likely if the test is simple enough to perform in-house rather than at specialist reference laboratories. The method you choose will depend on why you wish to type the isolates, as well as logistical factors such as cost, speed, and skill involved.

To interpret the data generated by *S. aureus* typing methods, it is essential to understand how *S. aureus* genomes vary, how *S. aureus* populations are structured, how they are evolving, and how stable genomes are. This has become possible in the last few years because of whole genome sequencing projects, large epidemiological studies with molecular typing methods, and whole genome comparative studies with multi-strain microarrays.

23.3 How Do *S. aureus* Isolates and Their Genomes Vary?

S. aureus populations are continually evolving in response to antibiotics, various hosts, and immune attack. *S. aureus* isolates can be grouped into dominant lineages and some minor lineages. In humans, approximately 10 lineages predominate; they are clonal complexes (CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, and CC51 [10]). Each lineage is very different from the others and is evolving independently. Each lineage has a unique and stable combination of hundreds of genes, notably the genes encoding surface proteins [11].

Within each lineage, minor variations in the core genome can occur. Single-nucleotide polymorphisms (SNPs) can occur, and this is the basis of MLST typing into ST rather than CC/lineage groups (see below). One whole genome sequencing study has estimated that in hospitals SNPs may occur on average once every 6 weeks

[12]. Other studies in patients have identified dozens of SNPs developing in the infecting isolate during the course of chronic infection; often they are selected because they enhance resistance to antibiotic therapy [13, 14].

The second major way that *S. aureus* strains vary is their carriage of MGEs. MGEs include bacteriophage, *S. aureus* pathogenicity islands (SaPI), plasmids, transposons, and staphylococcal cassette chromosomes (SCCs). These elements often encode important toxins, virulence factors, and antibiotic resistance genes [15]. Based on sequencing and microarray studies, most strains carry between 3 and 12 MGEs. MGEs are highly variable, each being composed of a mosaic of gene fragments found in other MGEs, but they can be grouped into families based on their major replicative machinery [16]. Each MGE has unique properties, but many are highly unstable and move into and out of bacteria at extremely high frequency [17, 18]. MGE movement can also be documented during the course of infection within a single patient [19–21]. Examples of the genes found on bacteriophage and SaPI are toxic shock syndrome toxin, enterotoxins A, B, C, K, and Q, chemotaxis inhibitory protein, staphylokinase, staphylococcal complement inhibitor, exfoliative toxins, and Panton–Valentine leukocidin (PVL) [15]. Plasmids and transposons can carry genes encoding resistance to antibiotics such as tetracyclines, aminoglycosides, macrolides, fusidic acid, mupirocin, β-lactams, and, more recently, glycopeptides [22].

The SSC element that encodes the mecA gene for resistance to β-lactamase-resistant β-lactams (such as methicillin) is an exception. Although it is mobile, it is transferred inefficiently and is generally stably integrated into the *orfX* gene. There are several types that are widespread, and the acquisition of each *SCCmec* type has been used as evidence of independent evolution of particular MRSA clones [23].

Since the *S. aureus* genome varies in distinct ways, it is very important to consider whether the typing method you wish to use can actually detect the variation you should be looking for and therefore that you are interpreting the results correctly. In fact, most typing methods are best at proving two isolates are unrelated. Proving two isolates are identical or closely related is impossible without whole genome sequencing and testing the stability of any changes, which is obviously not feasible for routine typing. Therefore, compromises must be made when choosing a method. Knowing your local *S. aureus* epidemiology can help enormously to identify which is the most suitable method.

23.4 Global Epidemiology

In humans, about 10 lineages of MSSA predominate, and all carriage strains seem capable of causing disease [15]. Increasingly, studies from outside of the UK suggest that there are geographical differences in the predominant *S. aureus* lineages [24–28]. Animals can also be carriers of *S. aureus* and can be infected. Companion animals such as dogs and cats are often affected by human lineages [29], while cows and other ruminants, and pigs have their own lineages, and chickens carry a variant of the CC5 lineage [30–32].

The biggest epidemiological shift in the last 50 years has been the acquisition of methicillin resistance due to the mecA gene carried on *SCCmec* elements [1]. *SCCmec* have moved into the lineages CC5, CC8, CC22, CC30, and CC45 and become successful in hospitals [23]. An interesting study of CC5 MRSA in Germany proved that the *SCCmec* element was moving into local CC5 MSSA isolates generating multiple new clones [33], although this may not be true for other lineages. There is a marked geographical difference in the distribution of lineages in different geographical areas, and in most hospitals, only one or two clones dominate [8, 34]. Some countries have reported shifts in the dominant lineages over time [35–37].

CA-MRSA emerged outside of hospitals and predominantly in the lineages CC1 and CC8. However, some less common lineages have acquired both the *SCCmec*IV and PVL toxin on a bacteriophage and spread rapidly, including ST80 and ST59 [38]. More recently, an MRSA clone associated with pig farming has emerged to cause infection in humans (CC398) [39].

The important message here is that there is substantial variation in *S. aureus* and MRSA types depending on geography, clinical setting, and time. It is therefore vital to understand your local epidemiology before choosing a method for typing and interpreting your results. For example, the major MRSA clones in UK hospitals are CC22 (MRSA-15) and CC30 (ST36, MRSA-16). When investigating an outbreak of MRSA in a UK hospital, a typing method that detects only lineage is not going to be very useful on its own, and methods that target MGEs (or SNPs) are also required.

23.5 Typing Methods

S. aureus typing methods are now all molecular and based on DNA sequence variation. They usually rely on the generation of a pure culture of *S. aureus* as the first step. In the clinical setting, this usually means that a specimen is plated onto a primary plate and then a single colony is chosen and plated again onto a fresh agar plate or is grown in broth. These two steps usually take 1 day each. The pure culture then has its DNA extracted—a relatively simple and rapid method suitable for PCR steps (see MLST) or the commercially available automated magnetic bead methods (e.g. MagNA Pure) [40]. Alternatively, if good-quality DNA is required for microarrays or if the DNA is to be stored, then Qiagen columns or the Edge system is recommended [19, 34]. In the clinical lab, if a rapid MRSA detection system using PCR is used (such as BD GeneOhm MRSA or Cephaid Xpert™ MRSA) [41], then the DNA extracted may be suitable—however, this DNA is not necessarily from a single bacterial isolate that can complicate data interpretation. Many typing methods rely on PCR to generate bands for sizing on agarose gels or for subsequent sequencing, and most standard methods are suitable. A step-by-step example is provided in Lindsay and Sung [42].

Ideally, typing methods should identify both lineage and carriage of a range of MGE. Most methods listed below achieve only one of these, and so they may be

used combined with another method. Microarrays and whole genome sequencing have the potential to do both, but microarrays have only recently been developed for routine typing and are still improving. Note that for local typing of an MRSA outbreak, such as in the hospital setting, there may be only one or two dominant clones of MRSA in the hospital, so a lineage method alone will not be sufficient to discriminate between variants.

23.5.1 Detection of Lineages

Lineage detection uses methods that target variation in the relatively stable genes that are conserved within lineages but vary significantly between lineages. MLST is the gold standard, but spa typing is almost as useful and uses only one-seventh of the sequencing reactions and is therefore easier and cheaper to perform. A simple PCR test has also been developed, and microarrays are also useful.

23.5.1.1 Multi-Locus Sequence Typing

MLST involves the PCR amplification and sequencing of seven "housekeeping" genes; these genes are found in all isolates, and the sequences are relatively conserved because these genes are involved in basic metabolism. However, point mutations and minor variations are found, and they correspond closely with lineage. For typing, the sequence of each gene is compared using a Web database (www.mlst.net), and each sequence variant has a unique number assigned [43]. Each strain is then defined by a series of seven numbers, which is then assigned a sequence type (ST) number. There are thousands of strains that have been sequence typed. If two isolates share at least five out of seven numbers, they are assigned to the same clonal complex (CC), and CC is essentially the same as lineage. This can be visualised using free software called eBURST [44].

MLST is an excellent method for assigning lineage. The significance of ST types within a lineage/CC is less clear. There are some cases where an ST type correlates strongly with a unique phenotype, such as the ST36 variant of CC30, which is unique to epidemic MRSA strains found in the UK that have spread to other parts of Europe and around the world [23]. In other cases, ST variants within a lineage do not seem to represent true evolutionary branches [11]. Possibly, this is because a single point mutation is sufficient to generate a different "ST type", which is not truly a new evolutionary branch, or because isolates within a lineage frequently exchange DNA [45].

MLST uses pure DNA, seven PCR reactions, and sequencing of each PCR product in both directions [43]. PCR is simple and cheap. If you are only typing a small number of strains and do not have your own sequencer, the sequencing reactions are outsourced and rapidly become expensive. Alternatively, if large numbers of strains are routinely MLST typed, a sequencing machine can be purchased or hired, and

bulk reagents used so the cost per strain is modest. The data are reproducible, but can be severely affected by sequencing errors leading to incorrect ST assignment, although lineage assignment would remain correct. The data are in a format that is easily reported and comparable with other laboratories. MLST is widely used by research laboratories and reference laboratories as the gold standard when publishing epidemiological data.

23.5.1.2 spa Typing

The *spa* gene encodes protein A, a protein anchored to the cell wall that binds non-specifically to the F_c portion of antibodies, and is found in all isolates of *S. aureus*. The “X” region in the C terminal end of the *spa* gene varies according to lineage, and isolates from different lineages have different amino acid combinations in multiple repeat patterns [46–48]. Specifically, most strains have an X region of between 2 and 18 different short sequence repeat regions (SSRRs), and each SSRR is around 24 bp each. For typing purposes, each unique SSRR sequence has been assigned an “r” number, e.g. r01, r02. Each *S. aureus* isolate is assigned a series of 2–18 “r” numbers in order, and then this defines the *spa* type number [48]. *spa* type numbers are reported in the format “t001,” “t002”, etc., and several thousands have been described so far. The *spa* typing method requires pure DNA, PCR amplification of a region of the *spa* gene, sequencing of the region in both directions, and comparison of the sequence to a public database, which contains all the SSRR type numbers and *spa* type numbers (www.spaserver.ridom.de) [48]. Software called BURP can be used to show the relatedness of each *spa* type [49].

spa typing is a reliable way to assign lineage. When assigning a *spa* type to a lineage, the matching MLST CC- or ST-type numbers are used, and for practical purposes tables of matching *spa* and MLST types are available (www.spaserver.ridom.de). Isolates of the same lineage have related *spa* types; for example, *spa* types of t001, t002, t003, and t010 all belong to lineage CC5 and have similar sequences of SSRR “r” numbers, varying only in minor deletions of an SSRR, duplications of an SSRR, or point mutations causing a change in an SSRR “r” number. *spa* type variation between isolates within the same lineage can be used for typing purposes, especially when investigating outbreaks. However, it cannot be assumed that two isolates within the same lineage with slightly different SSRR “r” numbers are epidemiologically unrelated. The stability of the *spa* region during outbreaks is relatively unknown, and *spa* variants do not always match variants identified using MLST [50, 51] or whole genome sequencing [52]. However, there are examples where lineage variants correlate well with epidemiological spread, suggesting there are occasions when variations of the gene are stable. Therefore, care should be taken when interpreting lineage “variants” to ensure that they are only considered significant when backed up with robust epidemiological data.

spa typing is reproducible [8], although sequencing errors are possible. The results are easily compared between laboratories using standard *spa* type numbers from the database. The cost of PCR is inexpensive, the equipment required is widely

available and inexpensive, and the reaction takes only a few hours. The equipment for sequencing is not standard, and as for MLST, if a lot of strains are to be typed, a sequencer should be purchased. Alternatively, for those typing a small number of isolates, commercial companies will sequence purified PCR products for a modest fee, and the results will be available within a week. Technically, this is not a difficult test, and only some experience interpreting the *spa* types is necessary.

spa typing is rapidly becoming the typing method of choice for reference and research laboratories as it is cheaper and simpler than MLST, equally discriminatory, reproducible, and easily reportable [51, 53]. Although *spa* typing relies on only one gene, which may be unstable, MLST typing also relies on variation in one of seven genes to assign a unique ST. *Spa* typing is most valuable when combined with other methods, especially those that can detect MGE variation [50, 51].

23.5.1.3 Microarray

Microarrays are solid supports (usually glass slides) with different DNA spots printed or synthesised onto the support in known order. Microarrays can carry only a few hundred spots or up to hundreds of thousands. They are used to interrogate complex mixtures of nucleic acid, including DNA from a pure culture for typing purposes. The bacterial DNA is labelled and hybridised to the slide so that unwound DNA strands with complementary base pairs will bind specifically to their matching spots, such that only those DNA spots representing genes found in the bacterial DNA will become labelled. The slide is then scanned to see which spots are labelled and the data analysed and compared to control strains and known populations. There are an infinite variety of microarrays depending on the number and types of DNA spots on the array, the solid support, labelling system, controls for the spots, hybridisation conditions, detection system, and data analysis methods [54]. For this reason, only microarrays that have been thoroughly validated for typing purposes using well-characterised (sequenced) strains of *S. aureus* should be used. For this discussion, there are two main types of microarrays to consider—comprehensive multi-strain *S. aureus* microarrays and those specifically designed for *S. aureus* typing.

Comprehensive multi-strain microarrays are excellent for assigning strains to lineage based on the presence or absence of hundreds of lineage-defining genes, such as surface proteins, regulators, hsdS variants, and exotoxins [11, 55]. In addition, they are an excellent method for identifying the presence and absence of a wide variety of MGE (see below). However, such microarrays are relatively expensive—arrays have to be purchased or printed, and the expense of labelling methods, especially the Cy dyes, restrict this technology to the research environment. Scanners are modestly expensive and so is software for analysing data. Microarray data are reproducible and take 1–2 days, but are very technically demanding, particularly to interpret the data. A large volume of data is generated so it is difficult to report, although it can be simplified to lineage and presence or absence of major toxins and resistance genes. For publication purposes, data should be MIAME compliant and deposited in a public database, which is a substantial amount of work [4, 56–60].

Such microarrays are invaluable for asking important epidemiological and biological questions about evolution, pathogenicity, host interactions, and the role of key genes in infection.

Several microarrays specifically designed for *S. aureus* typing have been developed [61–70]. At present, only one of them is designed specifically to identify lineage directly [71], but this should require only minor modifications to other microarrays, such as incorporating hsdS probes [34]. These microarrays currently focus on the presence or absence of putative virulence genes although the typing significance of these genes is often not clear. Companies also make it possible to design your own microarray to your own specifications, and this should be considered by reference laboratories who specialise in discriminating between *S. aureus* types in their local area. This is feasible now that so many *S. aureus* isolates have been fully sequenced [72]. Specific equipment for scanning microarrays and software for interpreting data are necessary, but these can be used for bacteria other than *S. aureus*. The cost of individual microarrays or "strips" of microarrays is low compared to other typing methods. The technique requires some expertise particularly in interpreting the data. *S. aureus* typing microarrays have the potential to be highly reproducible and reportable. There is likely to be a rapid improvement in technology and usefulness of these types of tests in the near future.

23.5.1.4 Restriction–Modification Test

The restriction–modification (RM) test is a simple PCR test to identify variants of the hsdS gene, which define the major MRSA lineages. All *S. aureus* isolates carry a type I restriction modification system called Sau1, and this consists of five genes, hsdR (restriction), two hsdM (modification), and two hsdS (specificity) [45]. RM systems identify foreign DNA at specific sequences, digest the DNA, and protect the host bacterium from bacteriophage. To protect the bacterium's own DNA, the same specific sequences are recognised and modified. *S. aureus* isolates belonging to the same lineage have the same hsdS sequences, but isolates from different lineages have different hsdS sequences. This means that DNA from different lineages is recognised as foreign and therefore exchanged between strains at lower frequency than within the same lineage. This controls the independent evolution of lineages and the spread of MGE [45, 73]. The test involves isolating DNA, up to three multiplex PCR reactions, which are separated on a standard agarose gel, and assigning a lineage based on a very simple test for band presence or absence.

The RM test is currently designed only to identify the major MRSA lineages, CC1, CC5, CC8/ST239, CC22, CC30, and CC45 [34, 73]. CC8 and ST239 can be distinguished by capsule-type PCR ([19], Cockfield and Lindsay unpublished) or by a PCR test based on the junction of the large recombination of a CC8 and CC30 strain that leads to the emergence of ST239 [74]. A new RM test that identifies ST398, the prevalent livestock-associated MRSA, has recently been validated [75]. The RM test will be expanded to include more lineages in the future.

RM typing is designed to be simple, rapid, and inexpensive. The results are reproducible and easily reported. It is particularly useful for typing very large numbers of isolates for epidemiological studies and can be easily automated or scaled up. It is a relatively new test, but its use is becoming more popular.

23.5.1.5 Pulsed-Field Gel Electrophoresis

PFGE was developed in the mid-1990s and was the first useful molecular test for typing *S. aureus*. It became popular with typing laboratories all over the world and is still widely used. PFGE patterns in experienced hands can provide useful information about dominant clones. PFGE cannot be used to define a lineage, but can provide clues if your local epidemiology is known, as well as some information about MGE distribution.

PFGE involves extraction of pure DNA using a specific process that catches the DNA in a plug of agarose, digestion of the DNA with SmaI, a site-specific restriction enzyme that cuts the *S. aureus* genome infrequently, and separation of the DNA fragments on an agarose gel in a specialised gel apparatus that applies electrical current in two or three directions in changing pulses and allows separation of much larger fragments than standard agarose gels. The gel is then stained, and the pattern of bands is photographed and compared using digital software.

PFGE identifies "patterns" of DNA fragments based on their size but does not identify what any of the fragments are. The insertion or deletion of an MGE is sufficient to cause a change to a PFGE band profile. For example, if a strain picks up a bacteriophage that integrates into the chromosome, and the phage contains two copies of the SmaI recognition site, the pattern will have two bands that differ in size and one extra band. Alternatively, a point mutation in a SmaI site can lead to changes in band size or number. In practice, it is acknowledged that during an outbreak situation, strains that vary in up to four bands are probably related [76], so interpretation of data is complicated. PFGE does not actually identify lineage and benefits from being combined with a method that determines lineage accurately [51]. However, in highly experience hands and when the local epidemiology is known, standard pattern types can be recognised as belonging to major clones, which in turn belong to known lineages. For example, the CDC in Atlanta uses PFGE to identify about 10 dominant clonal types of MRSA in the USA [77]. In the local typing situation (see below), where only one or two MRSA clones dominate, small variations in patterns can be useful. However, care should be taken to interpret the data, since relatively minor acquisition or loss of MGE can lead to significant band variation, yet this can occur in a single patient during the course of infection [20]. Furthermore, unrelated strains can have quite similar PFGE profiles [59].

PFGE requires investment in specific equipment, including a CHEF electrophoresis system, data documentation system, and software for comparing profiles. The reagents for each test are then modestly priced. However, the major drawback to PFGE is that the band patterns are difficult to reproduce unless the user is highly experienced. Therefore, standard protocols that carefully define every component

reagent and step have been developed in order to ensure that the data are reproducible and comparable between laboratories [78]. In cases where two strains from different gels are being compared, it may be necessary to run a further gel to compare them to each other. The patterns are not easily described without pictures and therefore it is difficult to compare results from different laboratories. The method itself is relatively labour intensive compared to other typing methods.

23.5.1.6 Other Methods

Prior to genetic tests such as PFGE, the major *S. aureus* typing method was bacteriophage typing [79]. Phage typing has not been formally compared to the newest typing methods, but it clearly provides clues to lineage and phage distribution without defining either. A set of bacteriophage, each grown on a specific *S. aureus* host strain, was spotted onto the strain to be tested and the pattern of phage lysis versus non-lysis to each phage used to discriminate between strains. Strains were typically classified into three or four major groups with the “international” typing set, and these groups probably correlate well with lineage, although this method cannot be used to define the lineage. In addition, significant variation within each group is seen, and this is probably correlated with the presence or absence of other bacteriophage in the host strain’s genome that prevent lysis with a related phage. There is still a lot that is not understood about how phage patterns are generated.

Phage typing is very inexpensive, rapid (overnight), and simple to perform and interpret. Only a few laboratories in the world still use phage typing and keep sets of phage. It is interesting that many modern MRSA are poorly lysed by the old phage sets [80, 81], making them less useful today.

Several other typing methods have been developed that have potential to rapidly and inexpensively identify lineage. Multiple locus variable tandem repeat analysis or variable number tandem repeats involves PCR of several surface protein genes that are then digested with restriction enzymes and the band sizes compared as patterns. It can detect lineages without defining them, but the correlation is not exact and comparing patterns between laboratories is difficult [82–84]. A similar method, staphylococcal interspersed repeat units, involves the sequencing of seven repeat regions scattered throughout the *S. aureus* chromosome. It is a useful method of separating strains of different lineage, but is not widely used and there are no support tools to assign lineages [85]. Amplified fragment length polymorphism involves the use of random primers to PCR random fragments of DNA and the patterns generated are compared. Although useful for rapid screening of large populations, it can identify only some of the lineages reliably [86]. A rapid version of MLST using mass spectrometry to detect variants has also been developed [87].

Other PCR tests are sometimes reported. The accessory gene regulator (agr) is the major regulator of toxin production in vitro, and there are four different types that can be discriminated by PCR: I, II, III, and IV [88]. However, their distribution correlates exactly with lineage [11], so the method is less sensitive than other

lineage-typing methods. Similarly, capsule type also correlates exactly with lineage [11, 89], and there are only two types: 5 and 8.

23.5.2 Detection of MGE

23.5.2.1 *SCCmec*

SCCmec typing is very popular as the *SCCmec* element is generally stable, and combined with a lineage detection method, it is useful for the identification of well-known epidemic MRSA types [23]. There are eight major types of *SCCmec*, types I–VIII, although new types and variants are increasingly being described. Within each type, there is variation, suggesting recombination and movement of elements such as transposons and plasmids embedded into the *SCCmec* element. Note that SCC elements without *mecA* genes exist, but are rare in methicillin-susceptible *S. aureus* [90]. Typing is dependent on PCR reactions that detect variation in the ccr recombinase genes and the *mecA* region, and use a potentially large number of primer pairs, but the methods are relatively straight forward, simple to perform, and inexpensive ([91, 92], www.staphylococcus.net/). This method is useful if you wish to assign an MRSA isolate to a major MRSA clonal type. However, it can be less useful in investigating local outbreaks, as the *SCCmec* elements are generally stable.

23.5.2.2 Microarray

Microarrays were described above for their ability to detect lineage. However, they are also particularly useful in detecting MGE variation because multiple MGE types and variants can be detected in a single experiment [93]. It is important to keep in mind that genes can be detected only if they are spotted on the microarray. Simple microarrays with a limited number of MGE spots are easier to interpret but less comprehensive than complex whole genome microarrays.

In general, there is no standard way to interpret microarray data. The presence or absence of a range of toxin and virulence genes, as well as antibiotic resistance genes found on MGE, is a logical place to start. Tables of various MGE types and their distribution into families based on integration genes and replication loci can also be very useful [16]. In practice, if two isolates have the same lineage and nearly identical combinations of MGE genes, as well as a strong epidemiological link, then they are likely to be the "same" strain. Two isolates that vary in genes likely found on only one MGE (e.g. one plasmid or one bacteriophage) and are also likely to be related but can be distinguished. Those with more variable MGE content are less likely to be epidemiologically related [4, 57–60].

23.5.2.3 PCR for Toxins and Other MGE Genes

There is currently no standard method for reporting the presence or absence of toxin genes encoded on MGE by PCR for typing purposes. However, increasingly, papers reference these reactions to justify their assignment of a strain to a particular clone or to investigate associations with particular diseases [94]. The most common example is the PVL toxin. PVL is relatively rare in *S. aureus*, but CA-MRSA nearly always carries it, and it is a useful marker for these strains [95]. Its role in CA-MRSA disease is controversial [96]. Other toxins with a potential role in pathogenicity can be useful to identify, especially if their detection has benefits for the patient or for understanding an outbreak, such as the food poisoning toxins [97], exfoliative toxins [98], and toxic shock syndrome toxin [95]. Such toxins may be unstable as they are found on MGE, and this should be considered when interpreting data, especially if only a few PCRs are used.

23.5.2.4 Antibiotic Resistance

Phenotypic antibiotic susceptibility testing is routinely performed in diagnostic laboratories on all *S. aureus* considered to be clinically significant. The results are generally available within 2 days of *S. aureus* diagnosis and are used to support the choice of antibiotic prescribed. There are highly standardised methods for identifying resistance using phenotypic methods, such as disc testing, automated broth testing, and E-tests, and published by organisations such as BSAC (http://www.bsac.org.uk/) and CLSI (formerly NCCLS). There is no standard method used by all diagnostic laboratories. Whichever method is chosen, it is very simple, inexpensive, and rapid.

Antibiotic testing played a part in the older typing methods prior to genetic tests. However, interpretation was difficult and unhelpful. Recently, we have been revisiting these tests in combination with lineage tests and find the combination much more useful. There is generally a wide range of resistance patterns to the major antibiotics, even within epidemic MRSA clones. It is therefore a potentially useful method for discriminating isolates during an outbreak (Budd and Lindsay, unpublished).

23.6 Other Staphylococcal Species

About 12 other species of staphylococci are found colonising the skin and mucous membranes of humans. They are all less virulent than *S. aureus*, but all are capable of causing disease, particularly in immunocompromised patients with prosthetic implants. They are often termed coagulase-negative staphylococci, and the most common skin coloniser and pathogen is *S. epidermidis*. Antibiotic resistance in all human staphylococcal species is common, especially methicillin resistance.

However, there is rarely a clinical reason to type *S. epidermidis* or other staphylococci in the hospital setting, as outbreaks are rare and disease is generally due to host immune factors. Large-scale epidemiological studies have been described recently, and an MLST typing method described for *S. epidermidis* [99]. *SCCmec* typing is also applied as many of these elements likely arose in coagulase-negative species [100]. Interestingly, disease isolates of *S. epidermidis* are predominantly from one particular lineage and have the ability to produce slime [101].

In dogs, the major species that causes disease is *S. pseudintermedius*. Multi-drug-resistant isolates are increasingly being described, and MLST, *spa* typing, PFGE, and *SCCmec* typing have also been developed [102].

23.7 Future

In the future, we are likely to see technologies developed that allow rapid and inexpensive typing of *S. aureus* in real time in the clinic. Already there are technologies that can identify pathogen species directly from clinical specimens using microarray detection [103–106]. While mixed populations of bacteria in specimens can make direct typing troublesome to interpret, these technologies are ideal for investigating isolated colonies on selective agar, even after minimal growth.

We are also likely to see improvement of mini-microarrays, particularly in designs that are adapted for different geographical regions. When investigating an MRSA outbreak in a hospital where only one or two MRSA predominate, the array needs to be focused on the MGE present in those clones. Hopefully, more commercial suppliers will enter the marketplace in the near future, automating methods and bringing down costs.

With rapid progress made in the DNA sequencing arena, there is also the possibility that *S. aureus* strain typing in the future will routinely involve whole genome sequencing [12]. This will be the ultimate typing tool and will require substantial progress in cost reduction and developing software able to rapidly compare and contrast the whole sequences of individual bacteria.

When typing can be performed in real time and the results fed back to the clinician, we are likely to see improved management of infections, better recognition of outbreaks, and faster responses to them. We will also recognise more correlations between types of strains and their associations with symptoms, prognosis, susceptible hosts, ability to spread, and geography. This is when we are likely to make the most progress in understanding *S. aureus* genome stability and evolutionary pressures.

23.8 Conclusions

At present, we are in a time of flux, with new typing technologies for *S. aureus* being developed but not fully taken up, and improved technologies on the hori-

zon. In the meantime, the choice of typing method is dependent on the question that is being asked. In general, a comprehensive typing of a strain requires the correct identification of lineage and some understanding of MGE variation. When typing to compare strains in an outbreak setting, such as MRSA in a hospital where only one or two clones dominate, a method that accentuates minor differences is preferred, and microarrays are becoming the method of choice. In the near future, these microarrays will be refined, and the technology will become less expensive and more rapid as the commercial market develops. In the future, the development of bench-top sequencing options, whole genome sequencing combined with customised data analysis software, will become the typing method of choice.

References

1. Lindsay JA (2010) Genomic variation and evolution of Staphylococcus aureus. Int J Med Microbiol 300:98–103
2. Nathwani D, Morgan M, Masterton RG, Dryden M, Cookson BD, French G, Lewis D, British Society for Antimicrobial Chemotherapy Working Party on Community-onset MRSA Infections (2008) Guidelines for UK practice for the diagnosis and management of methicillin-resistant Staphylococcus aureus (MRSA) infections presenting in the community. J Antimicrob Chemother 61:976–994
3. de Lassence A, Hidri N, Timsit JF, Joly-Guillou ML, Thiery G, Boyer A, Lable P, Blivet A, Kalinowski H, Martin Y, Lajonchere JP, Dreyfuss D (2006) Control and outcome of a large outbreak of colonization and infection with glycopeptide-intermediate Staphylococcus aureus in an intensive care unit. Clin Infect Dis 42:170–178
4. Edgeworth JD, Yadegarfar G, Pathak S, Batra R, Cockfield J, Wyncoll D, Beale R, Lindsay JA (2007) An outbreak of methicillin-resistant Staphylococcus aureus (MRSA)-ST 239 associated with a high rate of bacteremia. Clin Infect Dis 44:493–501
5. Schlievert PM, Shands KN, Dan BB, Schmid GP, Nishimura RD (1981) Identification and characterization of an exotoxin from Staphylococcus aureus associated with toxic-shock syndrome. J Infect Dis 143:509–516
6. Bergdoll MS, Crass BA, Reiser RF, Robbins RN, Davis JP (1981) A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome Staphylococcus aureus isolates. Lancet 1:1017–1021
7. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M-O, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 29:1128–1132
8. Aires de Sousa M, de Lencastre H (2003) Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J Clin Microbiol 41:3806–3815
9. Wulf M, Voss A (2008) MRSA in livestock animals-an epidemic waiting to happen? Clin Microbiol Infect 14(6):519–521
10. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, Peacock SJ, Smith JM, Murphy M, Spratt BG, Moore CE, Day NP (2003) How clonal is Staphylococcus aureus? J Bacteriol 185:3307–3316
11. Lindsay JA, Moore CE, Day NP, Peacock SJ, Witney AA, Stabler RA, Hussain SE, Butcher PD, Hinds J (2006) Microarrays reveal each of the ten dominant lineages of *Staphylococcus aureus* have unique combinations of surface-associated and regulatory genes. J Bacteriol 188:669–676

12. Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327:469–474
13. Mwangi MM, Wu SW, Zhou Y, Sieradzki K, de Lencastre H, Richardson P, Bruce D, Rubin E, Myers E, Siggia ED, Tomasz A (2007) Tracking the in vivo evolution of multidrug resistance in Staphylococcus aureus by whole-genome sequencing. Proc Natl Acad Sci USA 104:9451–9456
14. Howden BP, Stinear TP, Allen DL, Johnson PD, Ward PB, Davies JK (2008) Genomic analysis reveals a point mutation in the two-component sensor gene graS that leads to intermediate vancomycin resistance in clinical Staphylococcus aureus. Antimicrob Agents Chemother 52:3755–3762
15. Lindsay JA, Holden MTG (2006) Understanding the rise of the super bug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. Funct Integr Genomics 6: 186–201
16. Lindsay JA (2008) *S. aureus* evolution: lineages and mobile genetic elements (MGE). In: Lindsay JA (ed) Staphylococcus: Molecular Genetics. Caister Academic Press, UK
17. Lindsay JA, Ruzin A, Ross H, Kurepina N, Novick RP (1998) The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol 29:527–543
18. Ruzin A, Lindsay J, Novick RP (2001) Molecular genetics of SaPI1 – a mobile pathogenicity island in *Staphylococcus aureus*. Mol Microbiol 41:365–377
19. Moore PCL, Lindsay JA (2001) Genetic variation among hospital isolates of methicillin-sensitive *Staphylococcus aureus*: evidence for horizontal transfer of virulence genes. J Clin Microbiol 39:2760–2767
20. Goerke C, Matias y Papenberg S, Dasbach S, Dietz K, Ziebach R, Kahl BC, Wolz C (2004) Increased frequency of genomic alterations in Staphylococcus aureus during chronic infection is in part due to phage mobilization. J Infect Dis 189:724–734
21. Goerke C, Wolz C (2004) Regulatory and genomic plasticity of Staphylococcus aureus during persistent colonization and infection. Int J Med Microbiol 294:195–202
22. Jensen SO, Lyon BR (2009) Genetics of antimicrobial resistance in Staphylococcus aureus. Future Microbiol 4:565–582
23. Robinson DA, Enright MC (2003) Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 47:3926–3934
24. McDonald M, Dougall A, Holt D, Huygens F, Oppedisano F, Giffard PM, Inman-Bamber J, Stephens AJ, Towers R, Carapetis JR, Currie BJ (2006) Use of a single-nucleotide polymorphism genotyping system to demonstrate the unique epidemiology of methicillin-resistant Staphylococcus aureus in remote aboriginal communities. J Clin Microbiol 44:3720–3727
25. Vorobieva V, Bazhukova T, Hanssen AM, Caugant DA, Semenova N, Haldorsen BC, Simonsen GS, Sundsfjord A (2008) Clinical isolates of Staphylococcus aureus from the Arkhangelsk region, Russia: antimicrobial susceptibility, molecular epidemiology, and distribution of Panton-Valentine leukocidin genes. APMIS 116:877–887
26. Okon KO, Basset P, Uba A, Lin J, Oyawoye B, Shittu AO, Blanc DS (2009) Cooccurrence of predominant Panton-Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 Staphylococcus aureus clones in Nigerian hospitals. J Clin Microbiol 47:3000–3003
27. Ruimy R, Maiga A, Armand-Lefevre L, Maiga I, Diallo A, Koumaré AK, Ouattara K, Soumaré S, Gaillard K, Lucet JC, Andremont A, Feil EJ (2008) The carriage population of Staphylococcus aureus from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. J Bacteriol 190: 3962–3968
28. Chen H, Liu Y, Jiang X, Chen M, Wang H (2010) Rapid change of methicillin-resistant Staphylococcus aureus clones in a Chinese tertiary care hospital over a 15-year period. Antimicrob Agents Chemother 54:1842–1847

29. Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH (2005) J Antimicrob Chemother 56:692–697
30. Smith EM, Green LE, Medley GF, Bird HE, Fox LK, Schukken YH, Kruze JV, Bradley AJ, Zadoks RN, Dowson CG (2005) Multilocus sequence typing of intercontinental bovine Staphylococcus aureus isolates. J Clin Microbiol 43:4737–4743
31. Sung JM-L, Lloyd DH, Lindsay JA (2008) *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154:1949–1959
32. Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, Aarestrup FM (2010) spa type distribution in Staphylococcus aureus originating from pigs, cattle and poultry. Vet Microbiol 141:326–331
33. Nübel U, Roumagnac P, Feldkamp M, Song JH, Ko KS, Huang YC, Coombs G, Ip M, Westh H, Skov R, Struelens MJ, Goering RV, Strommenger B, Weller A, Witte W, Achtman M (2008) Frequent emergence and limited geographic dispersal of methicillin-resistant Staphylococcus aureus. Proc Natl Acad Sci USA 105:14130–14135
34. Cockfield JD, Edgeworth JD, Pathak S, Lindsay JA (2007) Rapid lineage typing of hospital MRSA. J Med Microbiol 56:614–619
35. Amorim ML, Aires de Sousa M, Sanches IS, Sá-Leão R, Cabeda JM, Amorim JM, de Lencastre H (2002) Clonal and antibiotic resistance profiles of methicillin-resistant Staphylococcus aureus (MRSA) from a Portuguese hospital over time. Microb Drug Resist 8: 301–309
36. Conceição T, Aires-de-Sousa M, Füzi M, Tóth A, Pászti J, Ungvári E, van Leeuwen WB, van Belkum A, Grundmann H, de Lencastre H (2007) Replacement of methicillin-resistant Staphylococcus aureus clones in Hungary over time: a 10-year surveillance study. Clin Microbiol Infect 13:971–979
37. Ellington MJ, Hope R, Livermore DM, Kearns AM, Henderson K, Cookson BD, Pearson A, Johnson AP (2010) Decline of EMRSA-16 amongst methicillin-resistant Staphylococcus aureus causing bacteraemias in the UK between 2001 and 2007. J Antimicrob Chemother 65: 446–448
38. Tristan A, Ferry T, Durand G, Dauwalder O, Bes M, Lina G, Vandenesch F, Etienne J (2007) Virulence determinants in community and hospital methicillin-resistant Staphylococcus aureus. J Hosp Infect 65(Suppl 2):105–109
39. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, Voss A, Wannet WJ, de Neeling AJ (2006) Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 5:26
40. Grisold AJ, Leitner E, Mühlbauer G, Marth E, Kessler HH (2002) Detection of methicillin-resistant Staphylococcus aureus and simultaneous confirmation by automated nucleic acid extraction and real-time PCR. J Clin Microbiol 40:2392–2397
41. Faria NA, Carrico JA, Oliveira DC, Ramirez M, de Lencastre H (2008) Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. J Clin Microbiol 46:136–144
42. Lindsay JA, Sung JM (2010) The RM test for determining methicillin-resistant Staphylococcus aureus lineages. Methods Mol Biol 642:3–11
43. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38:1008–1015
44. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG (2004) eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol 186:1518–1530
45. Waldron DE, Lindsay JA (2006) Sau1: a novel lineage-specific Type I Restriction-Modification system that blocks horizontal gene transfer into *Staphylococcus aureus*, and between *S. aureus* isolates of different lineages. J Bacteriol 188:5578–5585
46. Frénay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, Mooi FR (1996) Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis 15:60–64

47. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 37:3556–3563
48. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, Vogel U (2003) Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41:5442–5448
49. Mellmann A, Weniger T, Berssenbrügge C, Rothgänger J, Sammeth M, Stoye J, Harmsen D (2007) Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. BMC Microbiol 7:98
50. Hallin M, Deplano A, Denis O, De Mendonça R, De Ryck R, Struelens MJ (2007) Validation of pulsed-field gel electrophoresis and *spa* typing for long-term, nationwide epidemiological surveillance studies of Staphylococcus aureus infections. J Clin Microbiol 45:127–133
51. Mellmann A, Weniger T, Berssenbrügge C, Keckevoet U, Friedrich AW, Harmsen D, Grundmann H (2008) Characterization of clonal relatedness among the natural population of Staphylococcus aureus strains by using spa sequence typing and the BURP (based upon repeat patterns) algorithm. J Clin Microbiol 46:2805–2808
52. Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM (2005) Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. J Bacteriol 187:2426–2438
53. Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, Nübel U, Witte W (2008) spa Typing of Staphylococcus aureus as a frontline tool in epidemiological typing. J Clin Microbiol 46:574–581
54. Holden MTG, Lindsay JA (2008) Whole genomes: sequence, microarray and systems biology. In: Lindsay JA (ed) *Staphylococcus*: Molecular Genetics. Caister Academic Press, UK
55. Witney AA, Marsden GL, Holden MTG, Stabler RA, Husain SE, Vass JK, Butcher PD, Hinds J, Lindsay JA (2005) Design, validation and application of a seven-strain *Staphylococcus aureus* PCR product microarray for comparative genomics. Appl Environ Microbiol 71:7504–7514
56. Cassat JE, Dunman PM, McAleese F, Murphy E, Projan SJ, Smeltzer MS (2005) Comparative genomics of Staphylococcus aureus musculoskeletal isolates. J Bacteriol 187:576–592
57. Tang CT, Nguyen DT, Ngo TH, Nguyen TM, Le VT, To SD, Lindsay J, Nguyen TD, Bach VC, Le QT, Le TH, Le DL, Campbell J, Nguyen TK, Nguyen VV, Cockfield J, Le TG, Phan VN, Le HS, Huynh TS, Le VP, Counahan M, Bentsi-Enchill A, Brown R, Simmerman J, Nguyen TC, Tran TH, Farrar J, Schultsz C (2007) An outbreak of severe infections with community-acquired MRSA carrying the Panton-Valentine leukocidin following vaccination. PLoS One 2:e822
58. Vautor E, Cockfield J, Le Marechal C, Le Loir Y, Chevalier M, Robinson DA, Thiery R, Lindsay J (2009) Difference in virulence between Staphylococcus aureus isolates causing gangrenous mastitis versus subclinical mastitis in a dairy sheep flock. Vet Res 40:56
59. Stegger M, Lindsay JA, Sørum M, Gould KA, Skov R (2010) Genetic diversity in CC398 methicillin-resistant Staphylococcus aureus isolates of different geographical origin. Clin Microbiol Infect 16(7):1017–1019
60. Larsen AR, Goering R, Stegger M, Lindsay JA, Gould KA, Hinds J, Sørum M, Westh H, Boye K, Skov R (2009) Two distinct clones of methicillin-resistant Staphylococcus aureus (MRSA) with the same USA300 pulsed-field gel electrophoresis profile: a potential pitfall for identification of USA300 community-associated MRSA. J Clin Microbiol 47:3765–3768

61. Saunders NA, Underwood A, Kearns AM, Hallas G (2004) A virulence-associated gene microarray: a tool for investigation of the evolution and pathogenic potential of Staphylococcus aureus. Microbiology 150:3763–3771
62. Dunman PM, Mounts W, McAleese F, Immermann F, Macapagal D, Marsilio E, McDougal L, Tenover FC, Bradford PA, Petersen PJ, Projan SJ, Murphy E (2004) Uses of Staphylococcus aureus GeneChips in genotyping and genetic composition analysis. J Clin Microbiol 42:4275–4283
63. Monecke S, Ehricht R (2005) Rapid genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolates using miniaturised oligonucleotide arrays. Clin Microbiol Infect 11:825–833
64. Strommenger B, Schmidt C, Werner G, Roessle-Lorch B, Bachmann TT, Witte W (2007) DNA microarray for the detection of therapeutically relevant antibiotic resistance determinants in clinical isolates of Staphylococcus aureus. Mol Cell Probes 21:161–170
65. Zhu LX, Zhang ZW, Wang C, Yang HW, Jiang D, Zhang Q, Mitchelson K, Cheng J (2007) Use of a DNA microarray for simultaneous detection of antibiotic resistance genes among staphylococcal clinical isolates. J Clin Microbiol 45:3514–3521
66. Otsuka J, Kondoh Y, Amemiya T, Kitamura A, Ito T, Baba S, Cui L, Hiramatsu K, Tashiro T, Tashiro H (2008) Development and validation of microarray-based assay for epidemiological study of MRSA. Mol Cell Probes 22:1–13
67. Spence RP, Wright V, Ala-Aldeen DA, Turner DP, Wooldridge KG, James R (2008) Validation of virulence and epidemiology DNA microarray for identification and characterization of Staphylococcus aureus isolates. J Clin Microbiol 46:1620–1627
68. El Garch F, Hallin M, De Mendonça R, Denis O, Lefort A, Struelens MJ (2009) StaphVar-DNA microarray analysis of accessory genome elements of community-acquired methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 63:877–885
69. Vautor E, Magnone V, Rios G, Le Brigand K, Bergonier D, Lina G, Meugnier H, Barbry P, Thiéry R, Pépin M (2009) Genetic differences among Staphylococcus aureus isolates from dairy ruminant species: a single-dye DNA microarray approach. Vet Microbiol 133:105–114
70. Coombs GW, Monecke S, Ehricht R, Slickers P, Pearson JC, Tan HL, Christiansen KJ, O'Brien FG (2010) Differentiation of clonal complex 59 community-associated methicillin-resistant Staphylococcus aureus in Western Australia. Antimicrob Agents Chemother 54:1914–1921
71. Monecke S, Slickers P, Ehricht R (2008) Assignment of Staphylococcus aureus isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol 53:237–251
72. McCarthy AJ, Lindsay JA (2010) Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173
73. Sung JM-L, Lindsay JA (2007) *Staphylococcus aureus* that are hyper-susceptible to resistance gene transfer from enterococci. Antimicrob Agents Chemother 51:2189–2191
74. Feil EJ, Nickerson EK, Chantratita N, Wuthiekanun V, Srisomang P, Cousins R, Pan W, Zhang G, Xu B, Day NP, Peacock SJ (2008) Rapid detection of the pandemic methicillin-resistant Staphylococcus aureus clone ST 239, a dominant strain in Asian hospitals. J Clin Microbiol 46:1520–1522
75. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L (2011) Rapid PCR detection of Staphylococcus aureus clonal complex 398 by targeting the restriction-modification system carrying sau1-hsdS1. J Clin Microbiol 49:732–734
76. Bannerman TL, Hancock GA, Tenover FC, Miller JM (1995) Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of Staphylococcus aureus. J Clin Microbiol 33:551–555
77. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. J Clin Microbiol 41:5113–5120

78. Cookson BD, Robinson DA, Monk AB, Murchan S, Deplano A, de Ryck R, Struelens MJ, Scheel C, Fussing V, Salmenlinna S, Vuopio-Varkila J, Cuny C, Witte W, Tassios PT, Legakis NJ, van Leeuwen W, van Belkum A, Vindel A, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Muller-Premru M, Hryniewicz W, Rossney A, O'Connell B, Short BD, Thomas J, O'Hanlon S, Enright MC (2007) Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant Staphylococcus aureus strains: the HARMONY collection. J Clin Microbiol 45:1830–1837
79. Williams REO, Rippon JE (1952) Bacteriophage typing of Staphylococcus aureus. J Hyg (Lond) 50:320–353
80. O'Neill GL, Murchan S, Gil-Setas A, Aucken HM (2001) Identification and characterization of phage variants of a strain of epidemic methicillin-resistant Staphylococcus aureus (EMRSA-15). J Clin Microbiol 39:1540–1548
81. Murchan S, Aucken HM, O'Neill GL, Ganner M, Cookson BD (2004) Emergence, spread, and characterization of phage variants of epidemic methicillin-resistant Staphylococcus aureus 16 in England and Wales. J Clin Microbiol 42:5154–5160
82. Sabat A, Krzyszton-Russjan J, Strzalka W, Filipek R, Kosowska K, Hryniewicz W, Travis J, Potempa J (2003) New method for typing Staphylococcus aureus strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J Clin Microbiol 41:1801–1804
83. Malachowa N, Sabat A, Gniadkowski M, Krzyszton-Russjan J, Empel J, Miedzobrodzki J, Kosowska-Shick K, Appelbaum PC, Hryniewicz W (2005) Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, spa typing, and multilocus sequence typing for clonal characterization of Staphylococcus aureus isolates. J Clin Microbiol 43:3095–3100
84. Tenover FC, Vaughn RR, McDougal LK, Fosheim GE, McGowan JE Jr (2007) Multiple-locus variable-number tandem-repeat assay analysis of methicillin-resistant Staphylococcus aureus strains. J Clin Microbiol 45:2215–2219
85. Melles DC, van Leeuwen WB, Snijders SV, Horst-Kreft D, Peeters JK, Verbrugh HA, van Belkum A (2007) Comparison of multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) for genetic typing of Staphylococcus aureus. J Microbiol Methods 69:371–375
86. Hardy KJ, Ussery DW, Oppenheim BA, Hawkey PM (2004) Distribution and characterization of staphylococcal interspersed repeat units (SIRUs) and potential use for strain differentiation. Microbiology 150:4045–4052
87. Hall TA, Sampath R, Blyn LB, Ranken R, Ivy C, Melton R, Matthews H, White N, Li F, Harpin V, Ecker DJ, McDougal LK, Limbago B, Ross T, Wolk DM, Wysocki V, Carroll KC (2009) Rapid molecular genotyping and clonal complex assignment of Staphylococcus aureus isolates by PCR coupled to electrospray ionization-mass spectrometry. J Clin Microbiol 47:1733–1741
88. Gilot P, Lina G, Cochard T, Poutrel B (2002) Analysis of the genetic variability of genes encoding the RNA III-activating components Agric and TRAP in a population of *Staphylococcus* aureus strains isolated from cows with mastitis. J Clin Microbiol 40:4060–4067
89. Moore PCL, Lindsay JA (2002) Molecular characterisation of the dominant UK methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16. J Med Microbiol 51:516–521
90. Holden MTG, Feil EJ, Lindsay JA, Day NPJ, Enright MC, Foster TJ, Moore CE, Peacock SJ, Hurst L, Atkin R, Barron A, Bason N, Bentley SD, Chillingworth C, Chillingworth T, Churcher C, Clark L, Corton C, Cronin A, Doggett J, Dowd L, Feltwell T, Hance Z, Harris B, Hauser H, Holroyd S, Jagels K, James KD, Lennard N, Line A, Mayes R, Moule S, Mungall K, Ormond D, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Sharp S, Simmonds M, Stevens K, Whitehead S, Barrell BG, Spratt BG, Parkhill J (2004) Complete genomes of two clinical Staphylococcus aureus strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci USA 101:9786–9791

91. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51:264–274
92. Chen L, Mediavilla JR, Oliveira DC, Willey BM, de Lencastre H, Kreiswirth BN (2009) Multiplex Real-Time PCR for rapid Staphylococcal Cassette Chromosome mec Typing. J Clin Microbiol 47:3692–3706
93. Monecke S, Ehricht R, Slickers P, Wiese N, Jonas D (2009) Intra-strain variability of methicillin-resistant Staphylococcus aureus strains ST228-MRSA-I and ST5-MRSA-II. Eur J Clin Microbiol Infect Dis 28:1383–1390
94. Raulin O, Durand G, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, Etienne J, Laurent F (2010) Toxin profiling of Staphylococcus aureus strains involved in varicella superinfection. J Clin Microbiol 48:1696–1700
95. Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, Reverdy ME, Enright MC, Vandenesch F, Etienne J (2007) Global distribution of Panton-Valentine leukocidin–positive methicillin-resistant Staphylococcus aureus, 2006. Emerg Infect Dis 13:594–600
96. Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, Kreiswirth BN, DeLeo FR (2006) Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant Staphylococcus aureus disease? J Infect Dis 194:1761–1770
97. Lindsay JA (2011) The Staphylococci. In: Zhang W, Wiedmann M (ed) Genomics of foodborne pathogens, Springer, New York
98. Nakaminami H, Noguchi N, Ikeda M, Hasui M, Sato M, Yamamoto S, Yoshida T, Asano T, Senoue M, Sasatsu M (2008) Molecular epidemiology and antimicrobial susceptibilities of 273 exfoliative toxin-encoding-gene-positive Staphylococcus aureus isolates from patients with impetigo in Japan. J Med Microbiol 57:1251–1258
99. Miragaia M, Carriço JA, Thomas JC, Couto I, Enright MC, de Lencastre H (2008) Comparison of molecular typing methods for characterization of Staphylococcus epidermidis: proposal for clone definition. J Clin Microbiol 46:118–129
100. Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL (2003) Related clones containing *SCCmec* type IV predominate among clinically significant Staphylococcus epidermidis isolates. Antimicrob Agents Chemother 47:3574–3579
101. Ziebuhr W, Hennig S, Eckart M, Kränzler H, Batzilla C, Kozitskaya S (2006) Nosocomial infections by Staphylococcus epidermidis: how a commensal bacterium turns into a pathogen. Int J Antimicrob Agents 28(Suppl 1):S14–S20
102. Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L (2010) Clonal spread of methicillin-resistant Staphylococcus pseudintermedius in Europe and North America: an international multicentre study. J Antimicrob Chemother 65:1145–1154
103. Cleven BE, Palka-Santini M, Gielen J, Meembor S, Krönke M, Krut O (2006) Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. J Clin Microbiol 44:2389–2397
104. Wiesinger-Mayr H, Vierlinger K, Pichler R, Kriegner A, Hirschl AM, Presterl E, Bodrossy L, Noehammer C (2007) Identification of human pathogens isolated from blood using microarray hybridisation and signal pattern recognition. BMC Microbiol 7:78
105. Kurt K, Alderborn A, Nilsson M, Strommenger B, Witte W, Nübel U (2009) Multiplexed genotyping of methicillin-resistant Staphylococcus aureus isolates by use of padlock probes and tag microarrays. J Clin Microbiol 47:577–585
106. Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, Aittakorpi A, Laakso S, Lindfors M, Piiparinen H, Mäki M, Carder C, Huggett J, Gant V (2010) Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. Lancet 375:224–230