Usefulness of double locus sequence typing (DLST) for regional and international epidemiological surveillance of methicilin-resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections worldwide. To differentiate reliably among *S. aureus* isolates, we recently developed double locus sequence typing (DLST) based on the analysis of partial sequences of *clfB* and *spa* genes. In the present study, we evaluated the usefulness of DLST for epidemiological investigations of MRSA by routinely typing 1242 strains isolated in Western Switzerland. Additionally, particular local and international collections were typed by pulsed field gel electrophoresis (PFGE) and DLST to check the compatibility of DLST with the results obtained by PFGE, and for international comparisons. Using DLST, we identified the major MRSA clones of Western Switzerland, and demonstrated the close relationship between local and international clones. The congruence of 88% between the major PFGE and DLST clones indicated that our results obtained by DLST were compatible with earlier results obtained by PFGE. DLST could thus easily be incorporated in a routine surveillance procedure. In addition, the unambiguous definition of DLST types makes this method more suitable than PFGE for long-term epidemiological surveillance. Finally, the comparison of the results obtained by DLST, multilocus sequence typing, PFGE, Staphylococcal cassette chromosome *mec* typing and the detection of Panton-Valentine leukocidin genes indicated that no typing scheme should be used on its own. It is only the combination of data from different methods that gives the best chance of describing precisely the epidemiology and phylogeny of MRSA.

Keywords: *clfB*, molecular typing, MRSA, sequence typing, *Staphylococcus aureaus*, *spa*
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of nosocomial infections worldwide. Studies have shown that when a high proportion of MRSA has been encountered among *S. aureus*, one or few clones have been predominant [1]. Analysis of more than 3000 isolates from different countries demonstrated the existence of five major pandemic clones [1]. However, recent data have indicated that the geographical spread of MRSA over long distances is a rare event compared to the frequency with which the staphylococcal cassette chromosome *mec* (*SCCmec*) island has been acquired [2]. In addition, new strains that disseminate in the community [3] or in animals have recently emerged [4]. These strains have frequently been associated with specific biological characteristics (e.g. reduced susceptibility to antibiotics, enhanced virulence). These observations emphasize the importance of the surveillance of MRSA clones at regional and international levels.

During the last decade, the epidemiology of MRSA has mainly been analysed by pulsed field gel electrophoresis (PFGE). However, this method suffers from poor inter-laboratory standardization and from potentially ambiguous results, especially when large number of isolates are analysed. In this context, several DNA-based sequence methods such as multilocus sequence typing (MLST) [5] and *spa*-typing [6] have been developed for the epidemiological identification and differentiation of *S. aureus* strains. Recently, we have

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developed the double-locus sequence typing (DLST) method based on the analysis of approximately 500 bp of the highly variable *clfB* and *spa* genes [7]. This method gives more types and is far more discriminatory than *spa*-typing, matches the high resolution of PFGE and shows good congruence with the results obtained by MLST [7–9]. The combination of high typeability and reproducibility with low cost, ease of use and unambiguous definition of types makes this method especially useful for epidemiological analyses.

In the present study, we used DLST, *SCCmec* typing, and Panton-Valentine leukocidin (PVL) detection to analyse 1242 strains isolated in Western Switzerland, as well as a panel of strains representing major Swiss and international clones. The study aims were: (i) to evaluate the usefulness of DLST in investigation of the molecular epidemiology of MRSA at a regional level; (ii) to check whether the results obtained by DLST were compatible with earlier results obtained by PFGE; and (iii) to check whether the results obtained by DLST enabled a comparison with international predominant clones.

Materials and methods

Setting

Medical laboratories and infection control programmes in the canton of Vaud, Switzerland, refer all MRSA isolates (from infected or colonized in- or outpatients) to our reference laboratory (University Hospital of Lausanne).

Isolates

All isolates recovered between January 2005 and December 2007 and sent to the reference laboratory were re-identified at the species level and checked for methicilin-resistance [disc diffusion antibiogram with oxacillin and/or agglutination test for detection of the PBP2′ protein (Slidex MRSA detection; bioMérieux, Marcy l'Etoile, France)]. In addition, antibiograms were performed using the disc diffusion method. One isolate per patient and per year was saved by the reference laboratory. If a change in the antibiogram was observed, the new phenotype was also saved. For each patient, demographic data were collected (name, age and sex), as well as the date and site of MRSA isolation. Isolates were stored and further processed in 96-well plates, thus reducing cost and working time.

Double locus sequence typing

DLST was performed according to a previously described method [7]. For each gene, an arbitrary number was given to each allele, and the combination of both *clfB* and *spa* alleles constituted the DLST type. It is important to note that although *spa*-typing and DLST investigate polymorphisms in the *spa* gene, both methods do not analyse the same regions of the gene. Therefore, the *spa*-alleles of these two methods are not identical. Although the comparison between DLST and *spa*-typing was outside the scope of the present study, the correspondence between *spa*-typing and DLST-*spa* alleles is given in the Supporting Information for comparison purposes.

SCC*mec* typing and detection of PVL encoding genes

SCCmec was typed according to the scheme of Kondo *et al.* [10]. PVL encoding genes *lukS-PV* and *lukF-PV* were co-amplified by PCR reactions, as previously described [11], with primers luk-PV-1 and luk-PV-2. The same extracted DNA was used for DLST typing and for *SCCmec* typing and PVL detection.

Clone definition

Whereas the DLST markers appear to be highly stable during local epidemiological investigations [7], they might undergo changes over a long period. Therefore, we also considered related genotypes in our definition of clones. Because the reconstruction of genetic relatedness is highly problematic with sequences containing repeats, we used an approach similar to the analysis of MLST data with the BURST (Based Upon Related Sequence Type) algorithm. We considered only allelic data and the relatedness was defined by clustering all DLST types sharing one of the two alleles (single locus variant, SLV). With this approach, a clone was defined by the cluster of SLV DLST types and its founder. This analysis was performed using EBURST (http://eburst.mlst.net/).

Comparison between the results obtained by DLST and PFGE

To assess the congruence between PFGE (used in our laboratory until 2005) and DLST, we analysed two datasets with both methods: (i) a retrospective selection of 189 isolates representing the major local PFGE clones isolated between 1997 and 2004 and (ii) the first 150 consecutive isolates of 2005.

Relatedness to international clones

A collection of 74 strains representing the major Swiss and international clones was analysed with DLST, MLST, *SCCmec* typing and the detection of PVL genes. MLST was performed as described previously [5].

Results

Local DLST analysis

In total, 1242 isolates (one per patient and per year) were recovered in the Vaud Canton of Switzerland between

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