Evaluating the usefulness of *spa* typing, in comparison with pulsed-field gel electrophoresis, for epidemiological typing of methicillin-resistant *Staphylococcus aureus* in a low-prevalence region in Sweden 2000–2004

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Abstract

The usefulness of *spa* typing was evaluated in relation to pulsed-field gel electrophoresis (PFGE), as a tool for epidemiological typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in a low-prevalence region in southern Sweden. Bacterial isolates from 216 MRSA cases, newly identified in 2000–2004, were studied. The isolates were obtained from infected patients (31%), and from colonized individuals found by screening (69%). In total, 49 *spa* types and 73 PFGE patterns were identified. The discriminatory power of *spa* typing was lower (94.9 ± 1.8%) than that of PFGE (97.3 ± 1.2%). For two *spa* types (t002 and t008) the Panton–Valentine leukocidin results added useful discriminatory information. The most common *spa* types were t044 (*n* = 31; four PFGE patterns), t002 (*n* = 24; 10 PFGE patterns), t067 (*n* = 12; four PFGE patterns), t050 (*n* = 12; one PFGE pattern), and t324 (*n* = 11; one PFGE pattern). Epidemiological investigations identified 91 single cases and 39 transmission chains, each involving two to 13 cases. All the transmission chains were held together both by *spa* and PFGE typing. Among the 91 single-case isolates, 33 *spa* types and 50 PFGE patterns were unique (matchless) at the time of identification. The low prevalence of MRSA, the low number of outbreaks, and the wide spectrum of strains due to frequent acquisitions abroad (49% of the cases), makes *spa* typing a useful complement to epidemiological investigations in our setting. However, we still recommend the continued use of PFGE for further discrimination of isolates with identical *spa* types when epidemiological data can not exclude possible transmission.

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Introduction

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is a cause for concern in many countries. In 2004, MRSA accounted for 25–50% of *S. aureus* septicaemia cases in southern Europe (EARSS; http://www.rivm.nl/earss). Although the MRSA rate is low in Sweden, the number of cases reported has been increasing each year (http://www.smittskyddsinstitutet.se/) [1]. Since 2000 MRSA has been notifiable in Sweden, both from infection and colonization. The Swedish Institute for Infectious Disease Control (SMI) continually surveys the prevalence and geographical spread of MRSA, and isolates from every newly reported case are referred to SMI for epidemiological typing.

Pulsed-field gel electrophoresis (PFGE) of macro restriction fragments is a commonly used technique for epidemiological typing of many bacterial species. Analysis of *Sma*I-digested DNA is still considered to be the reference standard for typing MRSA, and the method has proven superior to most other typing methods [2,3]. It was the first molecular typing method for which guidelines for interpretation of data were suggested [4]. Even though the effects of such standardization have been far-reaching, low interlaboratory comparability is still a problem [5].

Sequence-based typing methods such as multilocus sequence typing (MLST) [6], and *spa* typing, sequencing of the polymorphic X-region of the *S. aureus* protein A gene [7–9], have become frequently used alternatives to PFGE. This is due to the portability of sequence data and ease of

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exchanging results via databases available on the internet (http://www.mlst.net and http://www.spaserver.ridom.de). However, MLST, which relies on sequence analysis of fragments of seven housekeeping genes, is best suited for studying the evolutionary history of *S. aureus* [10,11]. The single locus analysis performed in *spa* typing gives information that has proven adequate in hospital settings [9,12,13].

The usefulness of *spa* typing, in comparison with PFGE, for early detection of transmission has, to our knowledge, so far not been evaluated in a low-prevalence region. In this study, we included all MRSA consecutively referred to the department of Clinical Microbiology and Immunology (CMI), Lund University Hospital, Sweden, during 2000–2004. Detailed epidemiological information, collected by the local infection control units, formed the basis of our evaluation. All isolates were further characterized by the presence or absence of genes coding for Panton–Valentine leukocidin (PVL). Representative isolates were subjected to MLST.

Materials and Methods

Bacterial isolates

A total of 216 MRSA isolates were analysed, each representing a case detected through clinical or screening samples referred to CMI from 2000 to 2004. The isolates were identified as MRSA by an in-house conventional PCR using the primers *nuc* N1-f (5′-GCGATTGATGGTGATACGGTT-3′), and *nuc* N2-r (5′-CAAGCCTTGACGAACTAAAGC-3′), adapted from Brakstad *et al.* [14], and *mec*A P3-f (5′-GGTA CTGCTATCCACCCTCAA-3′) and *mec*A P4-r (5′-CTTACT GCCTAATTCGAGTGCTA-3′). *Staphylococcus aureus* CCUG 35601 served as positive control for the *nuc*/*mec*A PCR and *S. aureus* ATCC 49775 for the *luk*S-PV/*luk*F-PV PCR.

Epidemiological investigations

CMI serves a region of *c.* 700 000 inhabitants. MRSA cases were categorized as either 'Infected' (cases with clinical infection) or 'Colonized' (cases found through screening). Cases that had recently (defined here as within the preceding 6 months) been employed or treated in a hospital or nursing home were defined as 'Healthcare related'. Cases with no such contacts were defined either as 'Community related' or 'Unknown' when a hospital connection could not be excluded. Recent immigration, or travel to a foreign country, was defined as 'Acquired abroad'.

Screening samples were routinely taken from (i) patients and medical staff who had recently been employed or treated in a hospital or nursing home outside Scandinavia, or in a hospital or nursing home in Scandinavia known to have MRSA patients, (ii) fellow patients and staff at the same ward(s) as healthcare-related MRSA cases, and (iii) household members of community-related cases. Samples were taken from the anterior nares, throat, perineum, skin lesions, intravenous and stoma sites and urine from catheterized patients. Newly discovered MRSA cases were diagnosed at or referred to the Departments of Infectious Diseases at Lund University Hospital, Kristianstad Central Hospital, or Helsingborg Hospital. Epidemiological investigations were performed in collaboration with the Hospital Infection Control Units.

spa Typing

spa Typing was performed at CMI, as described elsewhere [9], using the primers SPA1-f 5′-AAGACGATCCTTCGGT GA-3′ (adapted from [8]), and SPA2-r 5′-CACCAGGTTTAA CGACAT-3′ [7]. In 2004, SPA2-r was substituted by SPA3r 5′-AGCAGTAGTGCCGTTTGC-3′ (in-house). SPA-F9-f 5′-AACGTAACGGCTTCATCC-3′ was introduced when insufficient PCR product was obtained from *spa* type t355 isolates. These primers correspond to nucleotides 1094–1111, 1492–1475, 1533–1516 and 1067–1084, respectively, of *S. aureus* NCTC 8325-4 (GenBank J01786). All sequencing reactions were carried out using the ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA). The RIDOM STAPHTYPE® software (Ridom GmbH, Würzburg, Germany) was used for sequence analysis and assignment of *spa* types [9].

PFGE

PFGE analysis was performed at SMI according to standard procedures [15]. Briefly, *Sma*I-digested DNA was electrophoresed in 1% agarose in 0.5× TBE at 14°C for 23 h, using the CHEF Mapper XA system (Bio-Rad Laboratories, Hercules, CA, USA) set at 6 V/cm, with pulse times linearly increased from 5 s initial switch time to 60 s final switch time. *Sma*I-digested DNA from *S. aureus* NCTC 8325 was included as normalization standard on every gel. Ethidium bromide-stained gels were photographed over UV light with a charge-coupled device camera. The DNA banding patterns were included in a national MRSA–PFGE database, using the BIONUMERICS software, version 4.61 (Applied Maths NV, Sint-Martens-Latem, Belgium). Pair-wise similarities were calculated using the Dice coefficient, and the algorithm UPGMA (Unweighted Pair Group Method using Arithmetic averages) was used for constructing dendrograms. Position tolerance and optimization were both set at 1%. Each distinguishable banding pattern, within the size range 48–679 kb, was assigned a PFGE pattern name as in the following examples: (i) Bel EC-3a, UK E15, UK E16 (patterns indistinguishable

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