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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.

Keywords: MRSA, PFGE, *spa* typing, surveillance, Sweden
Original Submission: 29 April 2008; **Revised Submission:** 20 March 2009; **Accepted:** 23 May 2009
Editor: P. Tassios
Article published online: 15 July 2009
Clin Microbiol Infect 2010; **16:** 456–462
10.1111/j.1469-0691.2009.02881.x

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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′-GGTACTGCTATCCACCCTCAA-3′) and *mec*A P4-r (5′-CTTACTGCCTAATTCGAGTGCTA-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′-AAGACGATCCTTCGGTGA-3′ (adapted from [8]), and SPA2-r 5′-CACCAGGTTTAACGACAT-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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from DNA banding patterns of MRSA in the HARMONY collection [5]), (ii) SE03-5, SE03-5b (patterns not seen in the HARMONY collection, but in two or more MRSA isolates in the national collection; SE = Sweden, 03 = 2003, the year when MRSA with this PFGE pattern was first isolated in Sweden, 5 = a serial number for each unrelated SE03 pattern, b = an alphabetical suffix added to denote close relationship (>80% pair-wise Dice similarity) to SE03-5 or SE03-5a), (iii) Unique 001, Unique 002, etc. (patterns unrelated to any other pattern within the database).

MLST

MLST was performed, as previously described [6], on MRSA isolates representative of each SE prototype PFGE pattern. Sequence types (STs) were determined using the MLST database (http://www.mlst.net).

PVL genes

The PVL genes *lukS*-PV and *lukF*-PV were detected by PCR as described [16].

Statistical analysis

The discriminatory capacities of *spa* typing and PFGE were measured with Simpson's index of diversity [17] with 95% CI as previously described [18]. Concordance between the two methods was calculated as described elsewhere [19]. All calculations were performed using all single-case isolates (n = 91) and the first isolate in each epidemiologically defined transmission chain (n = 39). Chi-square test was used for comparison of proportions.

Results and discussion

The number of new cases, identified at CMI, increased each year, from 11 in 2000 to 81 in 2004, corresponding to an increase in incidence of *c.* 2–12 new cases per 100 000 inhabitants per year. This low incidence allowed for the implementation of an extensive infection control programme including early case-finding through extensive screening of patients with risk factors, and contact tracing combined with infection control measures such as isolation of MRSA cases. Generous inclusion of people in the community seeking medical care made culturing from this group feasible, and allowed early identification of new cases [1]. This may explain why a minority (66/216; 31%) of the isolates in this study were from infected cases. Skin and soft tissue infections dominated (53/66; 80%), followed by urinary tract infections and respiratory tract infections (6/66; 9% each). Only one isolate was obtained from a blood culture. The epidemiological investigations identified 105 (49%) cases as community related and 96 (44%) as healthcare related. The origin of acquisition could not be established for 15 (7%) cases, 12 of whom were immigrants who had recently visited their countries of origin. Infected cases were more frequently community related (44/66; 67%) than healthcare related (15/66; 23%), whereas colonized cases were more often healthcare related (81/150; 54%) than community related (61/150; 41%). The proportion of infected cases was significantly higher among community-related than healthcare-related cases, 42% vs. 16% (p <0.001). Half of the cases (107/216; 49%) had acquired their MRSA abroad, from a wide range of countries (Fig. 1).

Among the 216 MRSA isolates, we identified 49 *spa* types and 72 PFGE patterns (Fig. 1). One of the PFGE patterns, SE97-4, was represented by two DNA banding patterns differing in the high molecular weight part of the gel but indistinguishable within the normalized part of the gel. The concordance between *spa* and PFGE typing was 96% (both when using 72 and 73 PFGE patterns in the calculation), which is in agreement with other reports [20,21].

In total, 88 *spa* type/PFGE pattern combinations were recorded. t044/SE94-1 (ST80) was the most common combination (n = 27). t044 was also seen in combination with SE94-1b (n = 2), SE94-1d (n = 1) and SE94-1m (n = 1). A similar situation, with different PFGE patterns among t044

FIG. 1. Characteristics of 216 MRSA isolated from infected or colonized cases in a low-prevalence region in Sweden 2000–2004. An Unweighted Pair Group Method using Arithmetic averages (UPGMA) dendrogram shows the relatedness between the 72 pulsed-field gel electrophoresis patterns seen among the isolates. The scale shows percent similarity. a: (S), single case; (#n), transmission chain number. b: Country of acquisition when not Sweden: AF, Afghanistan; AU, Australia; BA, Bosnia-Herzegovina; CL, Chile; CO, Colombia; DE, Germany, EG, Egypt; ES, Spain; ET, Ethiopia; FR, France; FRY, Federal Republic of Yugoslavia until February 2003, thereafter Serbia-Montenegro; GM, Gambia; GR, Greece; HR, Croatia; HU, Hungary; IN, India; IQ, Iraq; IR, Iran; IT, Italy; KOR, Korea; LK, Sri Lanka; LV, Latvia; MT, Malta; PA, Panama; PH, the Philippines; PL, Poland; PRC, People's Republic of China; RU, Russia; SA, Saudi-Arabia; SO, Somalia; SY, Syria; TN, Tunisia; TT, Trinidad and Tobago; UAE, United Arab Emirates; UK, United Kingdom; USA, United States of America. c: 2 (#4); 2 (S), 5 (#5), 2 (#6), 2 (#7). d: 6 (S), 2 (#13), 2 (#14), 2 (#15), 2 (#16). e: DE, ES, FR, GR, HU, TT. f: 2 (S), 2 (#17), 2 (#18); 1 (S); 12 (#19); 2 (#20); 1 (S). g: 1 (S); 7 (S), 3 (#22), 3 (#23), 3 (#24), 1 (#25), 2 (#26), 3 (#27), 3 (#28), 2 (#29). h: FRY, IQ, MT, SA, SY, TN, UAE.

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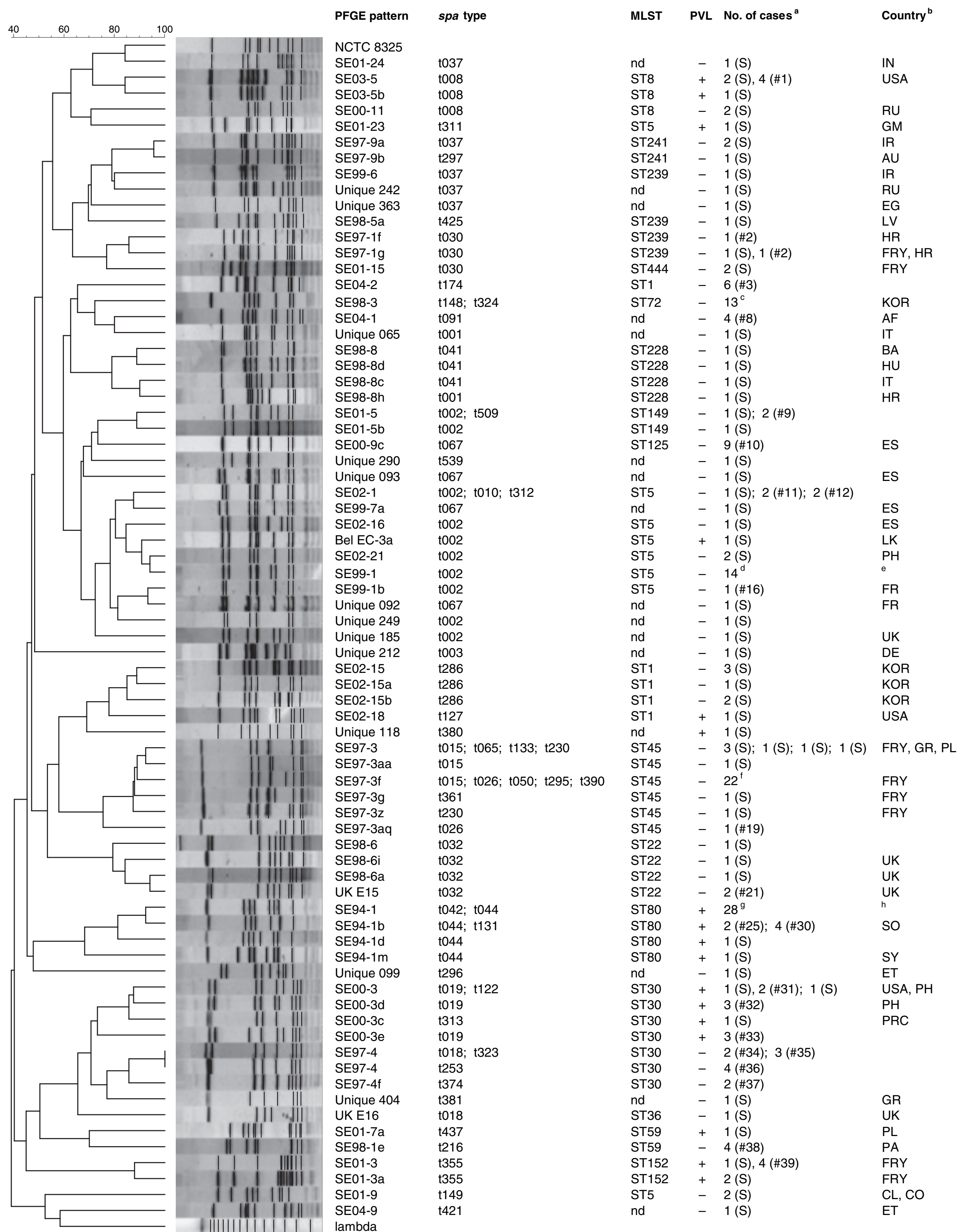
isolates, has been described in a Danish report [22]. SE94-1 was also described in combination with t042 (*n* = 1) and t131 (*n* = 4), which are both closely related to t044. All t044, t042 and t131 isolates were PVL-positive. ST80/t044 PVL-positive MRSA is widely spread, and often community acquired in Europe [22–26]. The majority of our t044/SE94-1



isolates were found in families with connection to the Middle East, and nearly all of the imported cases were from countries around the Persian Gulf.

t002/SE99-1 (ST5) was the second most common combination (*n* = 14). t002 was seen in another ten isolates showing nine different PFGE patterns that clustered with SE99-1 at 67% similarity in the UPGMA dendrogram. In total, 11 *spa* types and 23 PFGE patterns were recorded among 51 isolates belonging to MLST clonal complex 5 (CC5; here STs 5, 125, 149 and 228). This great variation was not surprising considering the evolution and worldwide spread of MRSA belonging to CC5 [10,11]. All but two of the isolates belonging to CC5 were PVL-negative. The two PVL-positive isolates were from cases with a history of recent travel to Asia (t002/Bel EC-3a) and Africa (t311/SE01-23).

The third most common combination (*n* = 12) was t050/SE97-3f (ST45). Twenty isolates had *spa* types (*n* = 8) and PFGE patterns (*n* = 5) closely related to t050 and SE97-3f, respectively. A majority of cases with ST45 isolates (24/32) had acquired their MRSA in Sweden, and none of them had connections with countries outside Europe. ST45 MRSA isolates have frequently been reported from many European countries [20–22,27]. All ST45 isolates were PVL-negative.

Also common during the study period were PVL-negative ST72 MRSA with PFGE pattern SE98-3, in combination with either *spa* type t324 (*n* = 11) or t148 (*n* = 2). These isolates were from children adopted from Korea, and from members of their Swedish families. In total, four transmission chains, no. 4 (2 cases), no. 5 (5 cases), no. 6 (2 cases) and no. 7 (2 cases), involved ST72 MRSA.

PVL genes were detected in 13/49 *spa* types and 17/72 PFGE patterns. In total, 31% of the isolates (25% of the single-case isolates and 38% of the transmission-chain isolates) were PVL-positive. Isolates with the same PFGE pattern were either all PVL-positive or all PVL-negative. The same homogeneity was seen for all *spa* types except t002 and t008, for which the PVL results added useful discriminating information. The PVL-positive MRSA variants found in our material have been reported from countries around the world [23,26–28]. The PVL-positive t008/SE03-5 (ST8; *n* = 7) MRSA was identified as a variant of USA300 [Hæggman, *et al.* 47th Intersci Conf Antimicrob Agents Chemother, abstract C2-143], the predominant community-associated MRSA in the USA [28].

Epidemiological investigations identified 39 transmission chains, involving two to 13 cases, and 91 single cases (Table 1 and Fig. 1). Twenty-three PFGE patterns and 25 *spa* types were identified among the 39 transmission chains. Twenty-eight of the 36 small chains (two to five persons) involved transmission within families only. Most of these family members (85/97) had had no known recent contact with the healthcare system. The remaining eight small chains (no. 11: t010/SE02-1, no. 12: t312/SE02-1, no. 14: t002/SE99-1, no. 15: t002/SE99-1, no. 20: t295/SE97-3f, no. 21: t032/UK E15, no. 25: t044/SE94-1, -1b, and no. 28: t044/SE94-1) involved one or more healthcare-related cases, and in five of them (nos 12, 20, 21, 25 and 28) the index case had a clinical infection. The three larger chains all involved healthcare-related cases. Chain no. 3 (t174/SE04-2, ST1) involved two patients and four personnel in a nursing home. Chain no. 10 (t067/SE00-9c, ST125) involved seven patients and two personnel in a hospital ward. The index patient in this chain had recently been hospitalized in Spain, where ST125 MRSA was already prevalent at that time [29]. The largest chain (no. 19) involved five patients and five members of the personnel in a nursing home, and three relatives of the personnel. Twelve of the isolates had PFGE pattern SE97-3f (ST45) and *spa* type t050 (repeat pattern 08-16-02-16-34-34-17-34-16-34), and one isolate, obtained from one of the relatives, differed slightly both by PFGE (SE97-3aq) and *spa* typing (t026, repeat pattern 08-16-34). This was the

TABLE 1. Concordance between epidemiological data and typing results for 125 MRSA isolates involved in 39 epidemiologically defined transmission chains

No. of cases per transmission chain	No. of transmission chains	No. of *spa* types	No. of PFGE patterns	No. of transmission chains comprising only isolates indistinguishable by	
				spa Typing	PFGE
2	19	14	12	19	18[a]
3	10	4	7	10	8[a]
4	6	6	6	6	6
5	1	1	1	1	1
6	1	1	1	1	1
9	1	1	1	1	1
13	1	2[b]	2[b]	0[b]	0[b]

[a]The three deviant transmission chains were chain nos 2, 16 and 25, all three comprising closely/possibly related PFGE patterns (Fig. 1).
[b]One isolate, given the same classification with both typing methods, differed slightly from the other twelve isolates in this transmission chain (no. 19).

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only transmission chain that included more than one *spa* type. More than one PFGE pattern was seen in another three chains, nos 2 (t030), 16 (t002) and 25 (t044). In total, 35 of the 39 chains were held together by PFGE and 38 by *spa* typing (Table 1). However, allowing closely and possibly related PFGE patterns within a transmission chain [4] and related *spa* repeat patterns, yielded full concordance between epidemiological data and typing results (Fig. 1).

When considering the 91 single-case isolates, the concordance between epidemiological data and typing results is not very high, although it is much higher for PFGE than for *spa* typing. The number of *spa* types and PFGE patterns that were unique (matchless) at the time of identification were 33 and 50, respectively.

In our study, the discriminatory power of PFGE was higher than that of *spa* typing (97.3 ± 1.2% vs. 94.9 ± 1.8%). Numerous studies, based on various collections of MRSA isolates, have been designed to compare different methods and their discriminatory power. Grundmann *et al.* [3] stated that PFGE was as discriminatory as MLST (97.6% vs. 95.7%). Two studies found *spa* typing to be as discriminatory as PFGE (97% vs. 96%) [21,30], whereas Stommenger *et al.* [20] found *spa* typing (96.9%) less discriminatory than PFGE (99.3%) but still better than MLST (93.1%). Even though the discriminatory power of *spa* typing was lower than PFGE in our study, we found the method useful as a complement to the detailed epidemiological investigations that were feasible in our low-prevalence setting, characterized by few outbreak situations and high numbers of acquisitions abroad. However, we still recommend continued use of PFGE, for example in reference laboratories, for further discrimination of isolates with identical *spa* types whenever a potential chain of transmission, which can not be disregarded based on available epidemiological data, needs to be investigated.

Acknowledgements

This study was presented in part at the 15th ECCMID, Copenhagen, Denmark, 2005. We thank the Hygiene department and P. J. Hugo Johansson at Lund University Hospital, and H. Elmrud and B. Carlsson, Kristianstad Hospital, for providing epidemiological data, and A. Lindmark, SMI, for skilful technical assistance performing PFGE.

Transparency Declaration

The authors have no conflict of interest to declare.

References

1. Stenhem M, Örtqvist Å, Ringberg H *et al.* Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Sweden 2000–2003, increasing incidence and regional differences. *BMC Infect Dis* 2006; 6: 30.
2. Tenover FC, Arbeit R, Archer G *et al.* Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbiol* 1994; 32: 407–415.
3. Grundmann H, Hori S, Enright MC *et al.* Determining the genetic structure of the natural population of *Staphylococcus aureus*: a comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. *J Clin Microbiol* 2002; 40: 4544–4546.
4. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
5. Murchan S, Kaufmann ME, Deplano A *et al.* Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* 2003; 41: 1574–1585.
6. Enright MC, Day NPJ, Davis CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38: 1008–1015.
7. Frénay HME, Bunschoten AE, Schouls LM *et al.* Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of the protein A gene polymorphism. *Eur J Clin Microbiol Infect Dis* 1996; 15: 60–64.
8. Shopsin B, Gomez M, Montgomery SO *et al.* Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999; 37: 3556–3563.
9. Harmsen D, Claus H, Witte W *et al.* 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* 2003; 41: 5442–5448.
10. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci USA* 2002; 99: 7687–7692.
11. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003; 47: 3926–3934.
12. Matussek A, Taipalensuu J, Einemo I, Tiefenthal M, Löfgren S. Transmission of *Staphylococcus aureus* from maternity unit staff members to newborns disclosed through *spa* typing. *Am J Infect Control* 2007; 35: 122–125.
13. Ruppitsch W, Stöger A, Braun O *et al.* Methicillin-resistant *Staphylococcus aureus*: occurrence of a new *spa* type in two acute care hospitals in Austria. *J Hosp Infect* 2007; 67: 316–322.
14. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J Clin Microbiol* 1992; 30: 1654–1660.
15. Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds, *Diagnostic molecular microbiology: principles and applications.* Washington, D.C: ASM Press, 1993; 563–572.
16. Lina G, Piémont Y, Godail-Gamot F *et al.* Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999; 29: 1128–1132.
17. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988; 26: 2465–2466.

©2009 The Authors

18. Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol* 2001; 39: 4190–4192.
19. Robinson DA, Hollingshead SK, Musser JM, Parkinson AJ, Briles DE, Crain MJ. The IS*1167* insertion sequence is a phylogenetically informative marker among isolates of serotype 6B *Streptococcus pneumoniae*. *J Mol Evol* 1998; 47: 222–229.
20. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, Witte W. Assignment of *Staphylococcus* isolates to groups by *spa* typing, SmaI macrorestriction analysis, and multilocus sequence typing. *J Clin Microbiol* 2006; 44: 2533–2540.
21. Hallin M, Deplano A, Denis O, De Mendonça R, De Ryck R, Struelens MJ. Validation of pulsed-field gel electrophoresis and *spa* typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. *J Clin Microbiol* 2007; 45: 127–133.
22. Faria NA, Oliveira DC, Westh H *et al.* Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. *J Clin Microbiol* 2005; 43: 1836–1842.
23. Vandenesch F, Naimi T, Enright MC *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: Worldwide emergence. *Emerg Infect Dis* 2003; 9: 978–984.
24. Witte W, Braulke C, Cuny C *et al.* Emergence of methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leukocidin genes in central Europe. *Eur J Clin Microbiol Infect Dis* 2005; 24: 1–5.
25. Urth T, Juul G, Skov R, Schønheyder HC. Spread of a methicillin-resistant *Staphylococcus aureus* ST80-IV clone in a Danish community. *Infect Control Hosp Epidemiol* 2005; 26: 144–149.
26. Tristan A, Bes M, Meugnier H *et al.* Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg Infect Dis* 2007; 13: 594–600.
27. Bartels MD, Boye K, Larsen AR, Skov R, Westh H. Rapid increase of genetically diverse methicillin-resistant *Staphylococcus aureus*, Copenhagen, Denmark. *Emerg Infect Dis* 2007; 13: 1533–1540.
28. Tenover FC, McDougal LK, Goering RV *et al.* Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *J Clin Microbiol* 2006; 44: 108–118.
29. Vindel A, Trincado P, Gómez E *et al.* Prevalence and evolution of methicillin-resistant *Staphylococcus aureus* in Spanish hospitals between 1996 and 2002. *J Clin Microbiol* 2006; 44: 266–270.
30. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among *Staphylococcus aureus* isolates: implication for use of a single marker to detect genetic micro- macrovariation. *J Clin Microbiol* 2004; 42: 792–799.

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