

Analyzing Multiclinality of *Staphylococcus aureus* in Clinical Diagnostics Using spa-Based Denaturing Gradient Gel Electrophoresis

Andreas Matussek, Lisa Stark, Olaf Dienus, Joakim Aronsson, Sara Mernelius,
Sture Lofgren and Per-Eric Lindgren

Linköping University Post Print

N.B.: When citing this work, cite the original article.

Original Publication:

Andreas Matussek, Lisa Stark, Olaf Dienus, Joakim Aronsson, Sara Mernelius, Sture Lofgren and Per-Eric Lindgren, Analyzing Multiclinality of *Staphylococcus aureus* in Clinical Diagnostics Using spa-Based Denaturing Gradient Gel Electrophoresis, 2011, Journal of Clinical Microbiology, (49), 10, 3647-3648.

<http://dx.doi.org/10.1128/JCM.00389-11>

Copyright: American Society for Microbiology

<http://www.asm.org/>

Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-71642>

**Analyzing multiclonality of *Staphylococcus aureus* in
clinical diagnostics using *spa*-DGGE**

Andreas Matussek^{1*}, Lisa Stark^{1,2*}, Olaf Dienus¹, Joakim Aronsson³,
Sara Mernelius^{1,2}, Sture Löfgren¹ and Per-Eric Lindgren^{1,2}

¹Department of Laboratory Medicine, Clinical Microbiology, Ryhov County Hospital,
Jönköping, ²Division of Medical Microbiology, Department of Clinical and
Experimental Medicine, Linköping University, Linköping,

³Department of Infectious Diseases, Ryhov County Hospital, Jönköping, Sweden

Key words: DGGE, *spa*, *Staphylococcus aureus*, MRSA, multiclonality

Running title: Multiclonality of *Staphylococcus aureus*

Corresponding author:

Lisa Stark

Department of Clinical Microbiology

Ryhov County Hospital

SE-551 85 Jönköping

Sweden

Phone: 0046-36-322302

Fax: 0046-36-322385

email: lisa.stark@lj.se

* The authors have contributed equally to this work

We present a novel Denaturing Gradient Gel Electrophoresis (DGGE) method which characterizes multiclonal communities of *Staphylococcus aureus*. The *spa* PCR-based DGGE method simultaneously separates strains that differ in only one base, thereby revealing multiclonal colonization and infections.

Staphylococcus aureus is causing a wide range of infections and is responsible for a considerable part of hospital-acquired infections. In 30 % to 70 % of healthy individuals *S. aureus* is a transient or persisting part of the residential flora (5, 10).

Cespedes et al. investigated the frequency of simultaneous nasal carriage of multiple *S. aureus* strains by picking three bacterial colonies from plates derived from each colonized individual. Less than 7 % of them were predicted to carry >1 strain (1). The simultaneous presence of an invasive and a carrier strain of MRSA in one individual, was reported by Soderqvist et al. (12). The issue of multiclonal colonization is important but conventional laboratory methods for detection of *S. aureus* are based on culture of a single colony. This might result in the identification of an antibiotic-susceptible commensal strain rather than a second more resistant strain, which may impair the antibiotic treatment and bias epidemiological conclusions.

We have developed a species specific DGGE method for *S. aureus*, utilizing *spa*, to characterize multiclonal colonization and infection. The novel assay was used to investigate a MRSA outbreak, revealing colonization with two different strains in some of the individuals.

spa-typing has been documented to be a useful tool in investigations of MRSA epidemiology (7) and for studies of *S. aureus* transmission (6). Thus, we based our DGGE method on *spa* and primer pairs, described by Kahl et al. (4), which were modified for DGGE analyzes by the attachment of a GC clamp (11) either at the forward or reverse primer. Annealing temperature and MgCl₂ concentrations for two primer combinations were optimized for PCR specificity and efficiency (data not shown).

Eight *S. aureus* isolates of known *spa*-types were acquired from the Microbiology laboratory, Ryhov County Hospital, Jönköping, Sweden. All isolates contained 10 *spa*-repeats (Table 1). The isolates were suspended in 200 µL PCR-grade water (Sigma-Aldrich, St. Louis, MO) and lysed for 10 minutes at 95 °C. DNA was purified using the MagAttract DNA Mini M48 Kit on BioRobot M48 (Qiagen, Hilden, Germany). The most stringent separation of the PCR-products (not shown) was achieved when the GC clamp (in bold) was attached to the forward primer *spa*-1113f-GC. The optimized PCR-reaction mixture contained 12.5 µL HotStar Mastermix (Qiagen), 1.5 mM MgCl₂ (Roche, Mannheim, Germany), 0.2 nmol forward primer *spa*-1113f-GC

(5'CGCCCGCCGCGCCCGCGCCCGTCCCGCCGCCCCCGCCCG

TAAAGACGATCCTTCGGTGAGC-3')

and 0.2 nmol reverse primer *spa*-1514r (5'-CAGCAGTAGTGCCGTTTGCTT-3') (TIBMOLBIOL, Berlin, Germany). The reaction conditions were 15 minutes at 95 °C, followed by 45 cycles of 30 s at 94 °C, 30 s at 61 °C and 60 s at 72 °C, with a final extension for 10 minutes at 72 °C.

The PCR-products were analyzed using a DCode universal mutation detection system (Bio-Rad Laboratories Inc, Hercules, CA). Polyacrylamide gradient gels (160 x 160 x 1 mm) composed of 37.5:1 acrylamide:bisacrylamide (7 %) and 1 x TAE (40 mM Tris-HCl, 20 mM sodium acetate, 1 mM EDTA [pH 8.3]) with 15 %–50 % denaturant, were cast with the aid of a gradient former (Bio-Rad Laboratories Inc). The gels were polymerized with 10 µL of TEMED and 173 µL of 10 % ammonium persulphate. 240 ng DNA were loaded to each well and the gels were run in 1 x TAE buffer at 62 °C for 14 h at 130 V. Gels were stained in 1x TAE buffer containing SYBRGold (Invitrogen, Paisley, UK) for 40 minutes and visualized in UV-transillumination using a CCD camera (LAS-3000, FujiFilm, Tokyo, Japan).

The detection limit was 20 gene copies of *spa* per reaction (not shown) and the method could discriminate PCR-products of the same length, from a mixture of eight strains (Fig 1). In the developed *spa*-DGGE assay it was possible to simultaneously amplify DNA of two different *spa*-types (t064 and t355, respectively) in a mixture with a concentration difference of 1:1000 (not shown). By using conventional cultivation methods, an extreme number of colonies would be required to achieve a comparable sensitivity to detect multiclonality.

An outbreak of MRSA occurred at a nursing home in 2009. Screening for possible colonization of patients (n=24), staff (n=50) and family members (n=6) of colonized staff was performed. Swab (Copan, Brescia, Italy) samples (n=229) from throat, anterior nares and groin, were cultured in broth and the presence of MRSA was verified in 37 broth samples from 12 individuals, by detection of *nuc* and *mecA* according to Nilsson et al. (9). One MRSA isolate from each individual was *spa*-typed as described previously (3, 4). Two different but closely related *spa*-types were isolated from the 12 individuals (4 patients, 4 staff and 4 relatives to staff) of the outbreak. Nine individuals were colonized with *S. aureus* of *spa*-type t015 and three with t069.

One mL of broth samples were centrifuged (10 000 g, 3 minutes) and DNA extraction was performed, as described above. When 28 available broth samples positive by culture for MRSA were analyzed by *spa*-DGGE, all samples were confirmed to contain either *spa*-type t015 or t069. Besides, in broths from three individuals, *spa*-types t015 and t069 were detected simultaneously (Fig 2). Dual colonization of MRSA was indicated in samples from the groin and the throat in one of these individuals, and in the throat of a second. The remaining samples from these

two individuals contained only *spa*-type t069. In the third individual the samples from throat and nares contained t069 and t015, respectively.

S. aureus multiclinality is rarely studied, although multiclinal infections occur and such infections may indeed affect the selection of antimicrobial therapy and might impede the treatment outcome of serious infections (2). Cespedes et al. showed by culture of three colonies from each sample that less than 7 % of the population was colonized by more than one strain in the anterior nares (1). However, their approach will probably only reveal major clones. Mongkolrattanothai K et al. found two genetically distinct *S. aureus* strains in 25 % of nasal and perianal swab samples from children when 4 to 15 colonies were picked from each culture (8). Thus, there is, using culture, still a risk of underestimating the diversity of *S. aureus*.

To conclude, we describe a sensitive, molecular method with high discriminatory power useful in clinical samples for multiclinal characterization of *S. aureus* colonization and infection. The method offers the potential to become a valuable epidemiological tool as well as a tool for the investigation of *S. aureus* infections.

We would like to acknowledge Andrea Johansson and Sofia Lundin for their technical assistance. This work was in part supported by the Swedish Society of Medicine, Futurum and the Research Council of South-East Sweden (FORSS).

REFERENCES

1. **Cespedes, C., B. Said-Salim, M. Miller, S. H. Lo, B. N. Kreiswirth, R. J. Gordon, P. Vavagiakis, R. S. Klein, and F. D. Lowy.** 2005. The clonality of *Staphylococcus aureus* nasal carriage. *J Infect Dis* **191**:444-452.
2. **Goerke, C., M. Gressinger, K. Endler, C. Breitkopf, K. Wardecki, M. Stern, C. Wolz, and B. C. Kahl.** 2007. High phenotypic diversity in infecting but not in colonizing *Staphylococcus aureus* populations. *Environ Microbiol* **9**:3134-3142.
3. **Harmsen, D., H. Claus, W. Witte, J. Rothganger, D. Turnwald, and U. Vogel.** 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.
4. **Kahl, B. C., A. Mellmann, S. Deiwick, G. Peters, and D. Harmsen.** 2005. Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of genetic change in *Staphylococcus aureus*. *J Clin Microbiol* **43**:502-505.
5. **Kluytmans, J., A. van Belkum, and H. Verbrugh.** 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* **10**:505-520.
6. **Matussek, A., J. Taipalensuu, I. M. Einemo, M. Tiefenthal, and S. Lofgren.** 2007. Transmission of *Staphylococcus aureus* from maternity unit staff members to newborns disclosed through *spa* typing. *Am J Infect Control* **35**:122-125.
7. **Melin, S., S. Haeggman, B. Olsson-Liljequist, M. Sjolund, P. A. Nilsson, B. Isaksson, S. Lofgren, and A. Matussek.** 2009. Epidemiological typing of methicillin-resistant *Staphylococcus aureus* (MRSA): *spa* typing versus pulsed-field gel electrophoresis. *Scand J Infect Dis* **41**:433-439.

8. **Mongkolrattanothai, K., B. M. Gray, P. Mankin, A. B. Stanfill, R. H. Pearl, L. J. Wallace, and R. K. Vegunta.** 2010. Simultaneous Carriage of Multiple Genotypes of *Staphylococcus aureus* in Children. *J Med Microbiol*.
9. **Nilsson, P., H. Alexandersson, and T. Ripa.** 2005. Use of broth enrichment and real-time PCR to exclude the presence of methicillin-resistant *Staphylococcus aureus* in clinical samples: a sensitive screening approach. *Clin Microbiol Infect* **11**:1027-1034.
10. **Nilsson, P., and T. Ripa.** 2006. *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. *J Clin Microbiol* **44**:3334-3339.
11. **Sheffield, V. C., D. R. Cox, L. S. Lerman, and R. M. Myers.** 1989. Attachment of a 40-base-pair G + C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. *Proc Natl Acad Sci U S A* **86**:232-236.
12. **Soderquist, B., and C. Berglund.** 2008. Simultaneous presence of an invasive and a carrier strain of methicillin-resistant *Staphylococcus aureus* (MRSA) in a family. *Scand J Infect Dis* **40**:987-989.

Table 1. *spa*-types and repeat succession of *S. aureus* strains used in the evaluation of the *spa*-DGGE method.

<i>spa</i> -type	Repeat succession
t015	08-16-02-16-34- 13 *-17-34-16-34
t050	08-16-02-16-34- 34 *-17-34-16-34
t008	11-19-12- 21 *-17-34-24-34-22-25
t064	11-19-12- 05 *-17-34-24-34-22-25
t002	26-23-17-34-17-20-17-12-17-16
t012	15-12-16-02-16-02-25-17-24-24
t355	07-56-12-17-16-16-33-31-57-12
t3061	07-21-17-34-13-34-34-13-33-13

*Repeat numbers 13 and 34 as well as 5 and 21 differ in only one base, respectively.

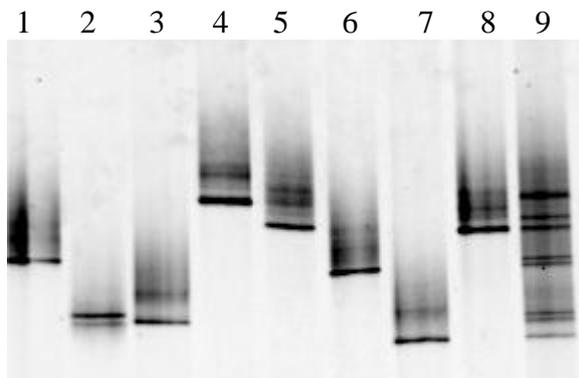


Figure 1. *spa*-DGGE analysis of the eight different strains (lanes 1 to 8; t064, t355, t012, t3061, t050, t008, t002, t015 respectively, lane 9; a mixture of equal amounts of eight PCR-products).

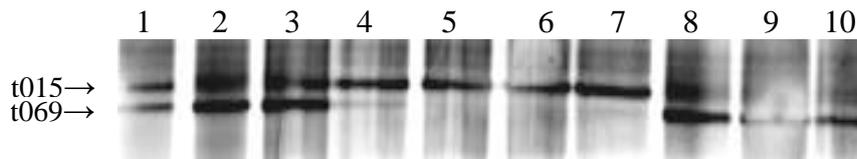


Figure 2. Broth samples from the outbreak containing MRSA of *spa*-types t015 or t069 were analyzed by the *spa*-DGGE method. In samples from two individuals both *spa*-types, t015 and t069, were detected (lanes 2 and 3). In lanes 4 to 7 the samples contain *spa*-type t015 and in lanes 8 to 10, the *spa*-type t069. In lane 1 PCR-products from both *spa*-types, t015 and t069, are mixed and analyzed as a control.