

THE CURRENT ROLE OF PULSED-FIELD GEL ELECTROPHORESIS IN METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) TYPING AND THE RETROSPECTIVE IDENTIFICATION OF OUTBREAKS^a

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The objective of this paper was to investigate whether retrospective pulsed-field gel electrophoresis (PFGE) of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates at two-year intervals is suitable and sufficient to demonstrate changes in the clonal composition of MRSA isolates and to identify previously undetected local outbreaks. PFGE patterns of 400 MRSA isolates were collected between 2004 and 2008 at the University of Rostock Hospital in Germany, and were used to assess the prevalence of MRSA clones at different time points. Only minor changes were detected. The combined analysis of all isolates that were collected per year reduced the time needed to perform this laborious procedure. The retrospective identification of outbreaks may require shorter intervals. Improved infection prevention and control measures prevented further outbreaks in previously affected hospital departments. In conclusion, PFGE at two-year intervals is sufficient to detect changes in the clonal composition of local MRSA isolates. If time for identification is important during outbreak investigations, more rapid methods with a similarly high discriminatory power such as *spa* typing should be used.

Keywords: pulsed-field gel electrophoresis, PFGE, methicillin-resistant *Staphylococcus aureus*, MRSA, typing, outbreak, epidemic clone

Introduction

Pulsed-field gel electrophoresis (PFGE) is a reliable tool for comparing methicillin-resistant *Staphylococcus aureus* (MRSA) isolates to known epidemic clones. This technique, however, requires specialised equipment and a high level of technical expertise, and has the additional disadvantage of poor inter-laboratory comparability. As a result, sequence-based typing methods such as *spa* typing or multilocus sequence typing (MLST) are often preferred. The objective of this study was therefore to investigate whether PFGE is still a useful MRSA typing method for the purpose of infection prevention and control in a hospital setting. For this reason, the prevalence of endemic MRSA clones at the University of Rostock Hospital in Germany was assessed using MRSA strains isolated between 2004 and 2008. Six MRSA clones are considered endemic in Rostock, i.e. the ‘Northern German’ clone (*spa* type t051; sequence type ST247; clonal complex CC8), the ‘Southern German’ clone (t001; ST228; CC5), the ‘Han-

nover’ clone (t009; ST254; CC8), the ‘Berlin’ clone (t004, t038, t065; ST45; CC45), the ‘Barnim’ clone (t032, t022, t005; ST22; CC22), and the ‘Rhine-Hesse’ clone (t002, t045; ST5; CC5) as defined by the Robert Koch Institute (RKI, Wernigerode, Germany) (Table 1), which is the National Reference Centre for Staphylococci in Germany. A further objective of this study was to assess the usefulness of PFGE data for retrospectively identifying local outbreaks and modifying the infection prevention and control strategies of the hospital departments affected.

Materials and methods

Strain collection

Methicillin-resistant *S. aureus* (MRSA) isolates are routinely collected and stored for 5 years at the Institute of Medical Microbiology of the University of Rostock Hospital. Only the first isolates that were obtained from new

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Table 1. Phylogenetic classification of MRSA clones with known endemicity in Rostock (Germany) according to the National Reference Centre for Staphylococci Robert Koch Institute, Wernigerode (Germany)

Clones	'Northern German'	'Southern German'	'Hannover'	'Berlin'	'Barnim'	'Rhine-Hesse'
Corresponding <i>spa</i> types	t051	t001	t009	t004, t038, t065	t005, t022, t032	t002, t045
Corresponding sequence types	ST247	ST228	ST254	ST45	ST22	ST5
Corresponding clonal complexes	CC8	CC5	CC8	CC45	CC22	CC5

MRSA cases in 2004, 2006, and 2008 were included in the study. Copy strains were excluded. Screening was performed during the entire study period in accordance with the recommendations of the National Reference Centre for Infectious Diseases (Robert Koch Institute) [1].

Pulsed-field gel electrophoresis

MRSA isolates from each patient were analysed using pulsed-field gel electrophoresis (PFGE) as described elsewhere [2, 3]. Visual inspection was performed on the basis of the criteria established by Tenover et al. [4]. The results were analysed with GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium; settings: Dice coefficient, UPGMA, 0.5% optimisation, 1.0% position tolerance).

Outbreak identification

MRSA distribution in the various hospital departments and outbreaks were assessed retrospectively. An outbreak was defined as three or more different cases of nosocomial infection or colonisation by one MRSA clone per month. All repeated occurrences of an MRSA clone in a department were tracked, however, if the interval between occurrences did not exceed 2 months.

Statistical analysis

Non-numerical data were analysed by Fisher's exact test. Numerical data were analysed by Student's *t*-test with different variances using GraphPad InStat 3 and Microsoft® Excel software. Significance was accepted at $p < 0.05$. If the *p*-value was smaller than 0.01, the differences were considered highly significant. If the *p*-value was smaller than 0.001, the differences were considered very highly significant.

Results and discussion

Strain collection and distribution of epidemic clones

MRSA strains were collected inconsistently as a result of organisational problems in the routine laboratory. In total,

127 of 168 (76%) different isolates were collected and analysed in 2004, 97 of 165 (59%) in 2006, and 176 of 267 (66%) in 2008. The clinical isolates that were assigned to the 'Northern German' epidemic clone accounted for 2% ($n=3$) in 2004, 1% ($n=1$) in 2006, and 1% ($n=2$) in 2008. The isolates that clustered with the 'Southern German' epidemic clone accounted for 12% ($n=15$) in 2004, 7% ($n=7$) in 2006, and 3% ($n=6$) in 2008. The isolates that clustered with the 'Hannover' epidemic clone accounted for 1% ($n=1$) in 2004, 1% ($n=1$) in 2006, and 0% ($n=0$) in 2008. The clinical isolates that were assigned to the 'Berlin' epidemic clone accounted for 5% ($n=6$) in 2004, 1% ($n=1$) in 2006, and 2% ($n=4$) in 2008. The isolates that clustered with the 'Barnim' epidemic clone accounted for 15% ($n=19$) in 2004, 13% ($n=12$) in 2006, and 35% ($n=62$) in 2008. The isolates that were assigned to the 'Rhine-Hesse' epidemic clone accounted for 65% ($n=83$) in 2004, 76% ($n=74$) in 2006, and 58% ($n=102$) in 2008. One isolate that was found in 2006 could not be assigned to one of the six aforementioned epidemic clones and was identified by the National Reference Centre for Staphylococci (RKI) as belonging to the 'Vienna' epidemic clone (phylogenetic classification of the 'Vienna' epidemic clone: t037, t030; ST239; CC8) (Figs 1–3).

The differences in the MRSA incidences in 2004, 2006, and 2008 were highly significant ($p < 0.01$). Furthermore, there was a very highly significant increase ($p < 0.001$) in the percentages of the 'Barnim' clone from 2004 to 2006 and from 2006 to 2008, and a highly significant decrease ($p < 0.01$) in the percentages of the 'Southern German' clone from 2004 to 2008 and the 'Rhine-Hesse' clone from 2006 to 2008. No further significant changes were detected.

Identified outbreaks

Nine distinct outbreaks affecting three to six patients each were identified retrospectively. Of these, seven had already been known at the time of outbreak as a result of the investigations by the hospital department responsible for infection prevention and control. Four outbreaks occurred on surgical wards, three on medical wards, one on a nuclear medicine ward, and one on a neurological ward.

All four outbreaks that occurred on general surgical and surgical intensive care wards were detected in 2004 and were caused by the 'Rhine-Hesse' clone. In 2006, the

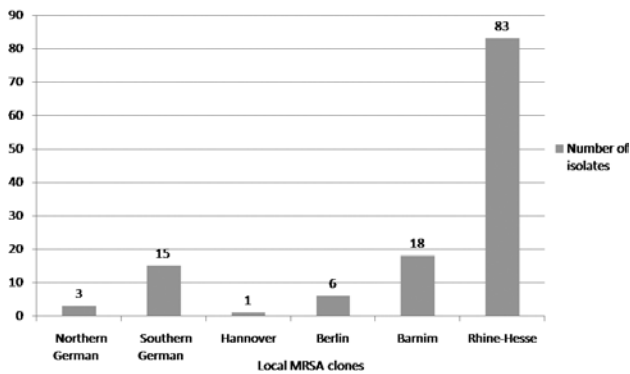


Fig. 1. Distribution of endemic MRSA clones at the University of Rostock Hospital in 2004

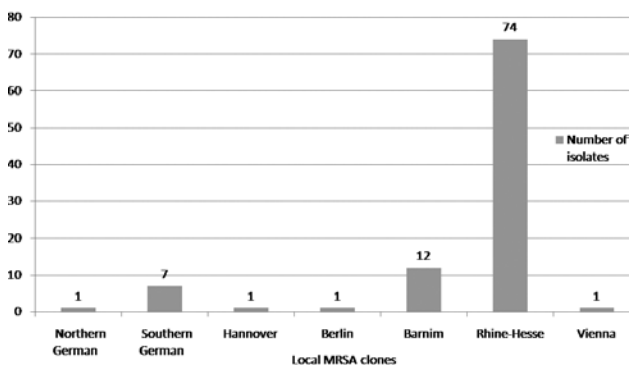


Fig. 2. Distribution of endemic MRSA clones at the University of Rostock Hospital in 2006

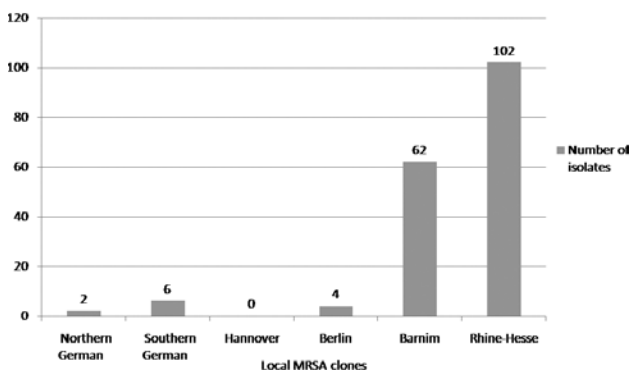


Fig. 3. Distribution of endemic MRSA clones at the University of Rostock Hospital in 2008

'Rhine-Hesse' clone was responsible for two outbreaks on general medical wards and one outbreak on a neurosurgical intensive care ward. In 2008, the 'Rhine-Hesse' clone was identified as the causative agent of two outbreaks on a general medical ward. The 'Barnim' clone was associated with an outbreak on a nuclear medicine ward.

In addition, several clones that did not meet the aforementioned outbreak criteria were repeatedly detected during the study period and occurred particularly in intensive care units, and, to a lesser degree, in general surgical and medical departments. Only the Barnim and 'Rhine-Hesse' clones were associated with cumulative increases in the number of isolates.

The cumulative increases that did not meet the criteria for an outbreak and two of nine outbreaks were identified only retrospectively. These findings show that the real-time or near-real-time detection of outbreaks by molecular typing requires more rapid procedures.

Past and present methods of DNA-based MRSA typing

The literature describes a wide variety of methods for typing *S. aureus* and MRSA including a number of DNA-based techniques. Many methods are affected by limitations regarding their discriminatory power, reproducibility, stability, or immediate availability in the event of an outbreak [5, 6].

Plasmid profile analysis was the first molecular method of studying the epidemiology of MRSA. Its discriminatory power is, however, limited by the influences of the complex three-dimensional structure of native plasmids on the mobility of plasmids in gel and the ability of bacteria to spontaneously lose or acquire plasmids [5]. Despite its disadvantages, plasmid profile analysis is still in use today [7].

More recent methods are based on the analysis of chromosomal DNA such as the interpretation of DNA restriction patterns and Southern blotting followed by hybridisation with DNA probes [5]. These developments culminated in the first successful PFGE analysis that was described for yeast DNA in 1984 [8]. The main disadvantage of PFGE is that it is a time-consuming method and the results are not available for several days [9].

The retrospective typing of the MRSA-isolates at the University of Rostock Hospital between 2004 and 2008 was exclusively performed by PFGE. The assignments to *spa* types or sequence types and resulting clonal complexes were based upon the phylogenetic classification by German National Reference Centre for Staphylococci (Table 1).

Polymerase chain reaction (PCR)-based methods of typing MRSA include PCR-restriction fragment length polymorphism (PCR-RFLP), PCR ribotyping, arbitrarily primed-PCR (AP-PCR) also known as typing by random amplified polymorphic DNA (RAPD), which is performed with short primers (up to ten bases) that have no known homology to genome sequences of the target organism, and the repetitive palindromic extragenic elements PCR (rep-PCR). The discriminatory power of PCR-based approaches, however, is poorer than that of PFGE, especially when it comes to investigating an endemic circulation of clones [5, 9]. Commercially available rep-PCR-based typing systems are available as well, such as the DiversiLab microbial typing system (bioMérieux, Nuertingen, Germany) [10]. The advantage of commercial systems is that they provide rapid results and are easy to use [11]. Another PCR-based typing method is multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA). Repeat-containing DNA fragments are amplified by multiplex PCR, and the resulting band patterns in agarose gels are

compared [12, 13]. High concordance between MLVA results and multilocus sequence typing (MLST) results can be achieved if appropriate cutoffs are used to distinguish between identical and different band patterns [14].

Sequence-based typing, especially MLST, correlates well with PFGE and facilitates supra-regional comparisons on the basis of numerical codes. The required sequencing of several genes is, however, laborious and expensive [5, 15]. For this reason, simpler techniques have been developed such as *spa* typing. This is a rapid and reproducible method based on analysing the polymorphic X region of the staphylococcal protein A (*spa*) gene, which consists of a variable number of 24 base pair repeats flanked by well-conserved regions [7, 16]. Sequence-based *spa* typing shows good correlation with PFGE and possesses a high discriminatory power [17, 18]. Another simple typing technique is double-locus sequence typing (DLST), which uses the *spa* and *clfB* genes, and shows an 88% concordance with PFGE [19].

The comparison of single-nucleotide polymorphisms (SNP) is also a sequence-based typing method [20] and provides the basis for real-time PCR or low-density array-based methods [21]. SNP analysis of three *Staphylococcus* enterotoxin genes (*set2*, *set5*, and *set7*) was reported to have even higher discriminatory power than MLST [22]. The use of sets of single-nucleotide polymorphisms in combination with binary markers, i.e. the presence or absence of certain pathogenicity factors, has been described for MRSA typing as well [23].

PFGE for the detection of changes in the clonal distribution of MRSA isolates and outbreaks

The long time required for results is one of the limitations of PFGE [9]. However, this disadvantage plays only a minor role if no real-time surveillance is intended, e.g. if isolates are not typed immediately after isolation but are collected and typed retrospectively at yearly intervals. If entire sample series are analysed at a later time, the time spent handling individual isolates can be reduced. Complex and expensive procedures such as sequencing are not required at all.

Our results suggest that if PFGE is performed at regular intervals, it is a suitable technique to detect local changes in the distribution of endemic clones in the hospital setting over the course of several years. During the study period, the 'Berlin', 'Hannover', and 'Northern German' clones were almost absent, and the 'Rhine-Hesse' clone was predominant at our hospital. In addition, we observed a relative decrease in the 'Southern German' clone and a marked increase in the 'Barnim' clone from 2004 to 2008. Since these changes took place gradually, typing intervals of 2 years appear to be sufficient to give a general picture of the situation.

The latter finding is in good concordance with the previously described rareness of geographical spreads

of MRSA clones over long distances or across cultural borders [24]. The increasing proportion of the 'Barnim' clone in Rostock reflects the epidemiological situation in Northern Germany. Two out of three MRSA isolates from the university hospital of Hannover (Northern Germany) could be assigned to the 'Barnim' clone in 2005 [25]. As recently described in 2011, the 'Barnim' clone was identified in 70% of MRSA isolates from nursing home residents in Brunswick (Northern Germany) [26]. An emergence of this clone was also monitored in Magdeburg in the centre of Germany between 2002 and 2005 [27]. In the meantime, the 'Barnim' clone is considered to be typical for hospital-acquired MRSA in the north of Germany. As published in 2008, the predominant clones in Eastern Saxony in the east of Germany comprise the 'Rhine-Hesse' clone, the 'Southern German' clone, the 'Barnim' clone, and the 'Berlin' clone, while, e.g. the 'Hannover' was only infrequently isolated [28]. Thus the clonal composition in Rostock reflects both the emergence of the 'Barnim' clone in the north of Germany and the stable persistence of the 'Rhine-Hesse' clone in the east of the country. As published in 2009, the 'Rhine-Hesse' clone and the 'Berlin' clone are common in the Western German parts of the Euregio Meuse-Rhin (EMR) region, while – in addition to these clones – a higher clonal diversity was detected on the Dutch side. The predominant clonal complexes in the EMR region are CC5, CC8, CC30, and CC45 [29]. Another study identified the 'Barnim' clone as one of the predominant clones in the Dutch–German border area [30]. The 'Barnim' clone also emerged in 2006 near the borders of Belgium and Germany [31]. A differing distribution can be found in the south. While the 'Southern German' clone and the 'Rhine-Hesse' clone were frequent in Austria between 1996 and 2006, the 'Northern German' clone, the 'Berlin' clone, and the 'Barnim' clone were only occasionally isolated [32]. A great genetic diversity of MRSA isolates was observed at the university hospital Basel (Switzerland) between 2000 and 2005 [33].

In addition to epidemiological monitoring and even more important for the hygiene management of a hospital, PFGE allows for the confirmation of outbreak situations. SmaI macro-restriction-based PFGE is particularly useful for this purpose, because SmaI macro-restriction provides a higher discriminatory power than *spa* typing or multilocus sequence typing [34]. Several MRSA outbreaks and local cumulative increases in the number of different MRSA isolates were confirmed or identified retrospectively in different hospital departments. These findings allowed the infection prevention and control team to take appropriate measures, and provide relevant infection prevention and control training for clinical staff in order to prevent further outbreaks. Despite these measures, MRSA clones were repeatedly isolated in high-risk departments such as intensive care units. No further outbreaks, however, occurred in previously affected departments so that the infection control measures that were taken after retrospective PFGE analysis have apparently been effective.

Conclusions

A time interval of 2 years is sufficient for PFGE to detect changes in the clonal composition of local MRSA isolates. If time for identification is important during outbreak investigations, more rapid methods with a similarly high discriminatory power such as *spa* typing should be used. These methods can contribute to the early identification of outbreaks and the immediate institution of appropriate infection control measures.

References

- Gastmeier P, Witte W, Kola A, Mattner F, Vonberg RP, Ziesing S, Suerbaum S, Reischl U, Weist K, Wendt C: Management of MRSA Screening. *Epi Bull* 42, 385–389 (2005) [Article in German]
- Struelens MJ, Deplano A, Godard C, Maes N, Serruys E: Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. *J Clin Microbiol* 30, 2599–2605 (1992)
- Winters MA, Goering RV, Boon SE, Morin R, Sorensen M, Snyder L: Epidemiological analysis of methicillin-resistant *Staphylococcus aureus* comparing plasmid typing with chromosomal analysis by field inversion gel electrophoresis. *Med Microbiol Lett* 2, 33–41 (1993)
- Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33, 2233–2239 (1995)
- Trindade PA, McCulloch JA, Oliveira GA, Mamizuka EM: Molecular techniques for MRSA typing: current issues and perspectives. *Braz J Infect Dis* 7, 32–43 (2003)
- Weller TM: Methicillin-resistant *Staphylococcus aureus* typing methods: which should be the international standard? *J Hosp Infect* 44, 160–172 (2000)
- Wildemaue C, De Brouwer D, Godard C, Buysens P, Dewit J, Joseph R, Vanhoof R: The use of *spa* and phage typing for characterization of a MRSA population in a Belgian hospital: comparison between 2002 and 2007. *Pathol Biol (Paris)* 58, 70–72 (2010)
- Schwarz DC, Cantor CR: Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 37, 67–75 (1984)
- Strandén A, Frei R, Widmer AF: Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? *J Clin Microbiol* 41, 3181–3186 (2003)
- Ross TL, Merz WG, Farkosh M, Carroll KC: Comparison of an automated repetitive sequence-based PCR microbial typing system to pulsed-field gel electrophoresis for analysis of outbreaks of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 43, 5642–5647 (2005)
- Shutt CK, Pounder JI, Page SR, Schaecher BJ, Woods GL: Clinical evaluation of the DiversiLab microbial typing system using repetitive-sequence-based PCR for characterization of *Staphylococcus aureus* strains. *J Clin Microbiol* 43, 1187–1192 (2005)
- Malachowa N, Sabat A, Gniadkowski M, Krzyszton-Russjan J, Empel J, Miedzobrodzki J, Kosowska-Shick K, Appelbaum PC, Hryniewicz W: 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 (2005)
- Tenover FC, Vaughn RR, McDougal LK, Fosheim GE, McGowan JE Jr: Multiple-locus variable-number tandem-repeat assay analysis of methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* 45, 2215–2219 (2007)
- Pourcel C, Hormigos K, Onteniente L, Sakwinska O, Deurenberg RH, Vergnaud G: Improved multiple-locus variable-number tandem-repeat assay for *Staphylococcus aureus* genotyping, providing a highly informative technique together with strong phylogenetic value. *J Clin Microbiol* 47, 3121–3128 (2009)
- Enright MC, Day NPI, Davies CE, Peacock SJ, Spratt BG: Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 38, 1008–1015 (2000)
- Hallin M, Friedrich AW, Struelens MJ: *spa* typing for epidemiological surveillance of *Staphylococcus aureus*. *Methods Mol Biol* 551, 189–202 (2009)
- Narukawa M, Yasuoka A, Note R, Funada H: Sequence-based *spa* typing as a rapid screening method for the areal and nosocomial outbreaks of MRSA. *Tohoku J Exp Med* 218, 207–213 (2009)
- Petersson AC, Olsson-Liljequist B, Mörner H, Hægman S: 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. *Clin Microbiol Infect* 16, 456–462 (2010)
- Basset P, Senn L, Pred'hom G, Bille J, Fracnioli P, Zanetti G, Blanc DS: Usefulness of double locus sequence typing (DLST) for regional and international epidemiological surveillance of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 16, 1289–1296 (2010)
- McDonald M, Dougall A, Holt D, Huygens F, Oppedisano F, Giffard PM, Inman-Bamber J, Stephens AJ, Towers R, Carapetis JR, Currie BJ: 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 (2006)
- Stephens AJ, Huygens F, Inman-Bamber J, Price EP, Nimmo GR, Schooneveldt J, Munckhof W, Giffard PM: Methicillin-resistant *Staphylococcus aureus* genotyping using a small set of polymorphisms. *J Med Microbiol* 55, 43–51 (2006)
- Aguiar-Alves F, Medeiros F, Fernandes O, Gudziki Pereira RM, Perdreau-Remington F, Riley LW: New *Staphylococcus aureus* genotyping method based on exotoxin (*set*) genes. *J Clin Microbiol* 44, 2728–2732 (2006)
- Huygens F, Inman-Bamber J, Nimmo GR, Munckhof W, Schooneveldt J, Harrison B, McMahon JA, Giffard PM: *Staphylococcus aureus* genotyping using novel real-time PCR formats. *J Clin Microbiol* 44, 3712–3719 (2006)
- 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: Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 105, 14130–14135 (2008)

25. Chaberny IF, Bindseil A, Sohr D, Gastmeier P: A point-prevalence study for MRSA in a German university hospital to identify patients at risk and to evaluate an established admission screening procedure. *Infection* 36, 526–532 (2008)
26. Pflingsten-Würzburg S, Pieper DH, Bautsch W, Probst-Keppler M: Prevalence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in nursing home residents in northern Germany. *J Hosp Infect* 78, 108–112 (2011)
27. Ghebremedhin B, König B, Witte W, Hardy KJ, Hawkey PM, König B: Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. *J Med Microbiol* 56, 365–375 (2007)
28. Monecke S, Jatzwauk L, Weber S, Slickers P, Ehrlich R: DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. *Clin Microbiol Infect* 14, 534–545 (2008)
29. Deurenberg RH, Nulens E, Valvatne H, Sebastian S, Diessen C, Craeghs J, De Brauwer E, Heising B, Kraat YJ, Riebe J, Stals FS, Trienekens TA, Scheres J, Friedrich AW, van Tiel FH, Beisser PS, Stobberingh EE: Cross-border dissemination of methicillin-resistant *Staphylococcus aureus*, Euregio Meuse-Rhin region. *Emerg Infect Dis* 15, 727–734 (2009)
30. Köck R, Brakensiek L, Mellmann A, Kipp F, Henderikx M, Harmsen D, Daniels-Haardt I, von Eiff C, Becker K, Hendrix MG, Friedrich AW: Cross-border comparison of the admission prevalence and clonal structure of methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 71, 320–326 (2009)
31. Nulens E, Stobberingh EE, Smeets E, van Dessel H, Welling MA, Sebastian S, van Tiel FH, Beisser PS, Deurenberg RH: Genetic diversity of methicillin-resistant *Staphylococcus aureus* in a tertiary hospital in The Netherlands between 2002 and 2006. *Eur J Clin Microbiol Infect Dis* 28, 631–639 (2009)
32. Krziwanek K, Luger C, Sammer B, Stumvoll S, Stammler M, Sagel U, Witte W, Mittermayer H: MRSA in Austria – an overview. *Clin Microbiol Infect* 14, 250–259 (2008)
33. Fenner L, Widmer AF, Dangel M, Frei R: Distribution of spa types among methicillin-resistant *Staphylococcus aureus* isolates during a 6 year period at a low-prevalence University Hospital. *J Med Microbiol* 57, 612–616 (2008)
34. 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* 44, 2533–2540 (2006)