Capillary Electrophoretic Methods for Classification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Clones

Marie Horká,[a] Filip Růžička,[b] Alena Siváková,[b] Pavel Karásek,[a] Jiří Šalplachta,[a,*] Roman Pantůček,[c] and Michal Roth [a]

Addresses

[a]Institute of Analytical Chemistry of the CAS, Veveří 97, 602 00 Brno, Czech Republic

[b]Department of Microbiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

[c]Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

*Corresponding author:

Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 97, Brno, 60200, Czech Republic

email: salplachta@iach.cz

phone number: 00420 532 290 213

ABSTRACT

This study describes differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates belonging to different genotype groups by the combination of electrophoretic techniques, transient isotachophoresis and micellar electrokinetic chromatography. MRSA isolates were separated in fused silica capillary with roughened inner surface prepared by etching with supercritical water. Separation temperature together with the rinsing procedure of the capillary turned out to be the key factors of successful analysis. The individual genotype groups were baseline-resolved in forty minutes. Partial separation of the individual isolates within the groups was also observed. Relative standard deviations of the migration times of the isolate zones ranged from 0.32 to 0.79 %. In addition, capability of the developed CE method to concentrate and separate MRSA isolates in clinical samples was proved by the analysis of blood sample.

Keywords: capillary electrophoresis; classification; fused silica capillary; separation; *Staphylococcus aureus*; temperature

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Abbreviations: *agr,* accessory gene regulator typing; BGE, background electrolyte; CCM, Czech Collection of Microorganisms; EtOH, ethanol; FS, fused silica; MLST, multi-locus sequence typing; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; PCR, polymerase chain reaction; PSS, physiological saline solution; *S. aureus*, *Staphylococcus aureus*; SCC*mec,* Staphylococcal Cassette Chromosome *mec* (chromosomal island involved in methicillin resistance); SCW, supercritical water; *spa*, staphylococcal protein A gene; ST, sequence type; tITP, transient isotachophoresis.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that can cause a wide spectrum of nosocomial infections associated with significant morbidity, mortality and also high medical care costs [1]. The morbidity increases as the therapeutic efficacy of the available antibiotics decreases, which is related to the increased occurrence of multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) [2]. *S. aureus* produces many virulence factors that contribute to the higher incidence of staphylococcal infections. These factors include surface proteins involved in establishing an infection, secreted compounds necessary for an outbreak of the infection, and biofilm formation that enable bacteria to adhere to various surfaces and protects the bacterium against host defenses and antimicrobials [3,4].

Specific phenotypic and genotypic characteristics of MRSA may play an important role in the serious infections [5-7]. The genotypically similar MRSA isolates can be classified into individual groups (clones) on the basis of their genotype profiles. MRSA isolates belonging to the single genotype group can have different phenotypic characteristics [5,7]. Various methods are used for the genotypic and phenotypic characterization of MRSA isolates [5,8,9]. Pulsed-field gel electrophoresis (PFGE) macrorestriction profile, multi-locus sequence type (MLST), staphylococcal protein A (*spa*) type, staphylococcal cassette chromosome *mec* (SCC*mec*), accessory gene regulator (*agr*) group, and capsular polysaccharide type are associated with particular clonal complex [10]. Additional genotyping methods such as Polymerase Chain Reaction (PCR) gene typing, repetitive element sequence-based PCR, plasmid analysis, or toxin gene profiling are used [11]. These methods are relatively rapid with good reproducibility and repeatability [12]. Nowadays, most techniques can be replaced by whole genome sequencing but it is relatively expensive, time-consuming, and requiring considerable expertise in data analysis [13]. Despite many genotyping methods developed for MRSA typing, there is no single

rapid technique with sufficient discriminatory power. Generally, low discrimination ability, technical complexity, difficult interpretation of the results, and high analysis costs are among the main disadvantages of the genotyping methods. Phenotyping methods include pathogen-drug and pathogen-host interactions, serotyping, protein electrophoresis and surface protein shaving [8, 9, 14]. Cultivation methods are easy to perform, cheap, and readily available in the clinical laboratories. On the other hand, these methods are often time-consuming with poor discriminatory ability [9, 12, 15]. A promising phenotyping tool is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This technique has been used for a fast and low-cost microbial species identification [16,17]. Although several authors demonstrated the ability of MALDI-TOF MS to identify MRSA [18-20], other authors reported poor reliability of the MALDI-based methods excluding MALDI-TOF MS from the use in routine laboratory [21, 22].

Capillary electrophoretic methods (CE) have proven to be suitable alternative to commonly used microbiological methods for the analysis of bacteria including *S. aureus* [8, 23, 24]. In general, bacteria are separated on the basis of the differences in the electrophoretic cell mobilities, which depend on the charge of the outer cell surface [23]. Bacterial outer surface is electrically charged under most conditions as it carries various dissociable groups (amino acid residues, carbohydrate moieties, etc.). When an electric field is applied, bacterial cells move with a velocity related to their electrophoretic cell mobilities and, thus, their separation occurs [23]. Microbial cell electric properties can be characterized by zeta potential (ζ), which is related to the electrophoretic mobility of the cells. The value of ζ is strongly dependent on chemical and physical environmental factors [25]. Chemical composition of cell surface [26], composition of background electrolyte (BGE) including additives (e.g., polyethylene glycol, PEG, non-ionogenic surfactant Brij 35) [27-32], pH of the BGE, sample matrix, inner surface structure of the capillary (rough or smooth) [8, 24], and the separation temperature [33] are

among these factors. CE was utilized to differentiate between methicillin-susceptible *S. aureus* (MSSA) and MRSA strains in our previous studies.

In this study, capillary electrophoretic techniques were used for the differentiation of the genotypically different clones of MRSA. Simultaneously, the MSSA cells were used for comparison of the mobility change during the method optimization. The combination of polymer-enhanced transient isotachophoresis (tITP) [31] from a high conductivity matrix and sweeping of the charged cells in micellar electrokinetic chromatography (MEKC) [30-32] was used for this purpose. The analyses were performed in a fused silica (FS) capillary with a roughened part prepared by etching with supercritical water (SCW) [8, 24, 30, 31, 34-36].

2. Materials and methods

2.1. Chemicals

Most of the chemicals, including non-ionogenic detergent Brij 35 and ethanol (EtOH), were purchased from Sigma (St. Louis, MO). Polyethylene glycol (M_r 10 000, PEG 10 000) was obtained from Aldrich (Milwaukee, WI). All chemicals were of electrophoresis or analytical grade purity.

2.2. Preparation of etched fused silica capillaries

The experimental setup for preparation of etched capillaries has already been described [35]. This type of structure can be defined as a set of conical formations lying close together with a base of 150 nm and a height between 200 and 300 nm. For SCW etching, the ultra-pure water at a density of 0.298 g cm^{-3} was used, created in a short heated zone at 420 °C and 340 bar. The

linear rate of capillary movement was set to 55 mm min^{-1} and each point of silica surface was etched for a period of 600 ms. Commercial FS capillaries, 50 μm i.d. and 360 μm o.d., were delivered by Agilent Technologies (Waldbronn, Germany). Purification of water consisted of a single distillation followed by reverse osmosis (Ultra Clear UV, SG Wasseraufbereitung und Regenerierstation GmbH, Barsbüttel, Germany). The resultant value of water conductivity was kept below 0.055 μS cm^{-1}, the total concentration of organic compounds was below 3 ppb, and the dissolved gases were removed by stripping with a gentle stream of helium (purity 4.8, Siad Czech spol. s r.o.).

2.3. Bacterial strains and growth conditions

The bacteria were isolated from various human or veterinary clinical samples from the Czech Republic and stored in Collection of Microbiology Institute, Masaryk University and St. Anne's University Hospital (Brno, Czech Republic). The MRSA isolates were genotypically characterized on the basis of MLST analysis [37] of the staphylococcal chromosome cassette containing SCC*mec* and the *spa* typing method that is based on sequencing of the polymorphic X region of the protein A gene (*spa*) [11]. The MSSA strain CCM 3953 was obtained from Czech Collection of Microorganisms (Brno, Czech Republic). The strain was used for comparison of the mobility change during the optimization procedure. Three isolates of each of three MRSA genotype groups were involved in this study: group A - ST8/t064/SCC*mec*-IV (FS195, FS196, FS197), group B - ST239/t037/SCC*mec*-III (FS198, FS199, FS200), and group C - ST225/t003/SCC*mec*-II (FS180, FS183, FS193). Type ST8/t064/SCC*mec*-IV was reported in veterinary samples (horses) from the United States and from some other countries [38]. ST239/t037/SCC*mec*-III is probably the oldest pandemic MRSA type, circulating in many countries since the 1970s. It is still frequently isolated worldwide. ST225/t003/SCC*mec*-II is a

typical nosocomial type and was predominant in the Czech Republic [39]. This type is also common in other European countries [40].

The tested isolates were cultivated on Mueller-Hinton agar (Oxoid, United Kingdom) at 37 °C for 24 h and the standardized bacterial suspensions were prepared as described previously [8]. Concentrations of the bacteria were determined by measurement of the optical density of the bacterial suspensions using a DU 520 UV-Vis spectrophotometer (Beckmann Instruments, Palo Alto, CA, USA) operating at 550 nm, according to the calibration curve, which was defined by the reference samples. Required sample concentrations were achieved by serial dilution of the prepared suspensions (10^7 cells mL^{-1}) with physiological saline solution (PSS, 0.85 % (w/v) NaCl). Human blood (group 0, Blood Bank of St. Anne's University Hospital in Brno, Czech Republic) was spiked with the bacterial cells. The preparation of blood samples prior to CE analysis was performed as described in our previous study [8]. Ten mL of the blood sample was spiked with 500 cells of each examined MRSA isolates of the genotype group A, FS195, FS196 and FS197, thus the concentration of each isolate in the prepared sample was 50 cells mL^{-1}. The sample was then processed according to the published procedure [8]. The pellet obtained after the purification steps, containing 500 cells of each isolate, was re-suspended in 100 μL of PSS and analyzed.

2.4. Safety considerations

The potentially pathogenic bacteria analyzed in this work belong to the risk group 2 of infectious agents. Although they can possibly cause a human disease, they do not present serious hazard to lab personnel under normal circumstances. Biological safety level 2 was maintained throughout the work with the living bacterial cultures. All experiments were

performed according to the instructions for the work with infective materials and the use of the latex gloves and regular disinfection of the laboratory equipment was maintained to avoid any contamination by microorganisms.

2.5. MEKC equipment and procedures

A laboratory-made apparatus [41] operated at a constant voltage (-20 kV on the detector side) supplied with a Spellman CZE 1000 R high-voltage unit (Plainview, NY, USA) was used for the electrophoretic experiments. The experiments were performed in the smooth (untreated) FS capillary and the FS capillary with roughened part at 20 or 25 °C. The FS capillary was 500 mm long, the distance between anode and the detection window was 350 mm. The FS capillary with roughened part was etched with SCW in the length of 250 mm from the anode side. The ends of the capillary and the electrodes were placed in 3-mL glass vials filled with BGE. The non-etched part of the capillary was orientated toward the cathode side. A LCD 2082 on-column UV-Vis detector (Ecom, Prague, Czech Republic), connected to the detection cell by optical fibers (Polymicro Technologies, Phoenix (AZ), USA), was operated at 235 or 280 nm. The detector signals were acquired and processed with the Clarity Chromatography Station (version 2.6.3.313, DataApex, Prague, Czech Republic). Each experiment was repeated ten times.

The BGE was composed of phosphate buffer (pH 7, 2×10^{-3} or 5×10^{-3} mol L^{-1}) and dissolved additives, EtOH (5–15 % (v/v)), PEG 10 000 (0.05–0.2 % (w/v)), and Brij 35 (0.05–0.3 % (w/v)). The examined MSSA and MRSA cells were suspended in PSS and vortexed using Thermomixer comfort (Eppendorf, Hamburg, Germany) at 25 °C for 2 min before analysis. The samples (100 nL) were injected into the capillary using the single-syringe infusion pump (Cole-Parmer, Vernon Hills, USA) at a flow rate of 1 μL min^{-1} from the anodic side. With respect to the blood sample, the dynamic adhesion of the cells from the prepared blood sample onto the

inner capillary surface was used [32]. In this case, 100 μL of the prepared sample (cell pellet suspended in 100 μL of PSS as described above) was dynamically injected into the capillary. Thiourea was used as a neutral marker of electroosmotic flow (EOF) in the measurements of the dependence of pH on the electroosmotic mobility, $\mu_{EOF,}$ at the temperatures of 20 or 25 °C.

Before each MEKC run, the capillary was rinsed with EtOH (2–10 min) and then back-flushed with BGE or 4×10^{-3} mol L^{-1} phosphate buffer, pH 7 (2–10 min). The rinsing was performed using the single-syringe infusion pump attached to the cathodic side of the capillary. Rinsing with each fluid consisted of two steps. At the first step, the capillary was rinsed with 10 μL of the particular fluid at a flow rate of 10 μL min^{-1}. At the second step, the particular fluid was left in the capillary for 1–9 min, i.e. the rest of the total rinsing time. This procedure ensured sufficient desorption of the potentially adhered bacterial cells from the roughened capillary surface before next analysis.

3. Results and discussion

3.1. MEKC separation of genotypically different groups of MRSA

Successful discrimination of MSSA and MRSA strains by CE in the SCW-etched FS capillary in our previous studies [8, 24] led to the idea to investigate the ability of CE for phenotyping of MRSA isolates. The experiments described in this study were designed and performed on the basis of our experience with CE analysis of various microorganisms utilizing SCW-etched FS capillary [8, 24, 32, 42, 43]. Three MRSA isolates (FS197, FS198, and FS180) belonging to different genotype groups, ST8/t064/SCC*mec*-IV, ST239/t037/SCC*mec*-III, and ST225/t003/SCC*mec*-II, designated group A, B, and C, respectively, and one MSSA strain were selected for the method development. The strains were separated by MEKC in both smooth FS

capillary and the SCW-etched FS capillary (Figure 1A). With respect to the different properties of the inner surfaces, different BGE composition (concentration of phosphate buffer and additives, PEG 10 000, Brij 35, and EtOH) had to be used for both types of capillaries for successful analysis of the examined bacteria. The temperature of separation and rinsing procedure were identical. In contrast to the smooth FS capillary, the SCW-etched FS capillary allowed separation of MSSA from MRSA strains. Further optimization of the experimental conditions (BGE composition, separation temperature, rinsing procedure) did not improve the separation of the analyzed MSSA and MRSA in the smooth FS capillary. Just a single peak was observed when the analysis was performed in the smooth FS capillary regardless of separation conditions used. The SCW-etched FS capillary, on the other hand, provided promising results as shown in Figure 1.

Roughened surface of the SCW-etched FS capillary had a positive effect on the separation of the MSSA strain from the MRSA isolates, although the EOF in the roughened capillary was significantly higher using phosphate buffer without additives as BGE. The lower the EOF is achieved the better separation of the bacteria can be observed. Figure 2 shows a comparison of the EOF in the smooth FS capillary (curve 1) and in the etched FS capillary (curve 3) at 25 °C. The use of BGE additives significantly reduced the EOF in the SCW-etched FS capillary (Figure 2, curve 4 vs. curve 3), but it was still higher than in the smooth FS capillary (Figure 2, curve 2). The experimental conditions were further optimized to lower the EOF. Decrease in additive concentration together with the shorter rinsing procedure in the case of SCW-etched FS capillary led to another significant decrease of the EOF (Figure 2, curve 5 vs. curve 4). The achieved EOF was approximately a fifth lower (at pH 7) than the EOF in the smooth FS capillary (Figure 2, curve 2). However, even significantly reduced EOF did not improve the separation and only single zone of the MRSA isolates was detected again (data not shown).

In an effort to separate the MRSA isolates, the temperature of separation was decreased to 20 °C. This led to a further decrease in the EOF (by fifth again at pH 7) at the same BGE composition as in the previous case (Figure 2, curve 6 vs. curve 5). Consequently, slightly better MRSA isolates separation (three overlapping peaks) was observed (Figure 1B). The increase in the concentration of Brij 35 to 0.3 % (w/v) in the BGE caused a slight reduction of the EOF (Figure 2, curve 7 vs curve 6) and, thus, even better separation of the MRSA isolates (Figure 1C). The isolate of the group A was separated from the co-eluting isolates of the groups B and C. The order of the isolate zones was determined experimentally by the standard addition method. Figure 1D shows complete separation of the MRSA isolates belonging to the individual genotype groups and also more distinctive separation of MSSA strain from the MRSA isolates (in comparison to the result shown in Figure 1A, etched capillary). This result was achieved when the concentration of Brij 35 was decreased to 0.1 % (w/v) and the rinsing procedure was changed again – 2 min with EtOH and 5 min with BGE. Under these conditions, a further drop of the EOF (Figure 2, curve 8) was observed which corresponded to half the EOF value at pH 7 in the smooth FS capillary at 25 °C (Figure 2, curve 2). Figure 1E shows the detected zones of the MRSA isolates of different genotype groups in detail. The MRSA isolates were analyzed individually for this purpose. The same migration times of the analyzed isolates were achieved as in the case of analysis of the isolate mixture. The obtained results confirmed that surface roughness together with separation temperature [33] and capillary rinsing method significantly affect bacterial separations. Beside the decrease in the EOF, the decrease in the temperature of separation also induced changes in diffusion and other physico-chemical properties of the entire separation system – BGE, roughened capillary surface and bacterial cells [44, 45]. This enabled the separation of different types of bacterial cells under the properly selected separation conditions. With respect to the achieved results, the following experiments were performed in the etched capillary at 20 °C.

3.2. MEKC separation of individual MRSA isolates within individual genotype groups

Three MRSA isolates of each genotype group (A – FS195, FS196, FS197; B – FS180, FS183, FS193; C – FS198, FS199, FS200) were selected for the next MEKC experiments. The BGE composition and other experimental conditions were essentially the same as in the experiments shown in Figure 1 D,E. The only change was the substitution of BGE with phosphate buffer (pH 7, 4×10^{-3} mol L^{-1}) for rinsing of the capillary, which led to further reduction in EOF (Figure 2, curve 9). Separation of the mixture of all nine selected MRSA isolates is shown in Figure 3. This result confirmed the ability of the CE method to discriminate individual genotype groups of MRSA. In addition, partial separation of the isolates within individual genotype groups was observed as can be seen in the inset figures (zone details) in Figure 3. Almost complete separation of the isolates was observed in the group A where narrow peaks of the individual isolates were detected. The order of the isolate zones was determined experimentally by the standard addition method again. Only partial separation of the isolates was observed in the groups C and B. The order of the isolates in the zones of the groups C and B was therefore not further determined. Further optimization of the separation conditions did not improve the separation of the individual isolates of the groups C and B. Because of the higher conductivity of the phosphate buffer used to rinse the capillary, the migration times of the isolate zones increased (which agrees with our previous results [32]), the detected zones were very narrow and their resolution increased. All the MRSA isolates were also analyzed individually. Summary of the sample analyses is given in Supplementary Table S1. The mean values of standard deviation (SD) and relative standard deviation (RSD) of the migration times of the bacteria zones calculated from all analyses were 13.25 s and 0.58 %, respectively. The proposed CE method enables reliable discrimination of the genotype groups of MRSA. In addition, the

obtained results suggest the possibility to separate individual isolates of a single genotype group.

3.3. MEKC separation of MRSA isolates of the genotype group A in a blood sample

Human blood sample was used in the next experiments to demonstrate the ability of the proposed CE method to analyze MRSA isolates in clinical samples. The blood sample was spiked with the examined MRSA isolates of the genotype group A (FS195, FS196 and FS197; concentration of each bacterium was 50 cells mL^{-1}), processed and analyzed by the optimized CE method (in the SCW-etched capillary at 20 °C). Schematic illustration of the procedure is shown in Figure 4. In this case, 100 μL of the prepared sample was dynamically injected into the capillary when the bacterial cells were adhered to the etched surface of the capillary and subsequently analyzed. The procedure enabling online concentration and separation of bacterial cells is described in detail in [32]. Three peaks of the MRSA isolates were detected upon the MEKC separation as shown in electropherogram in Figure 4. Their migration times were practically the same as in the analysis of the cultivated bacteria proving that the results obtained by this method are independent of the sample matrix. The recovery of the method was approximately 80 %.

4. Conclusion

MRSA isolates of three genotype groups differing in sequence type, *spa* type and type of staphylococcal chromosome cassette *mec* were separated in the FS capillary with a roughened inner surface. Experimental conditions were optimized to allow discrimination of the individual genotype groups, which was the main goal of this study. Especially the changes in separation

temperature and in the capillary rinsing procedure played the key role in the optimization process. Whole analysis can be done in 60 min including sample preparation and rinsing of the capillary. The developed method even enabled partial separation of the MRSA isolates within individual genotype groups indicating the effect of phenotypic differences between strains. However, their complete separation would require further extensive investigation. Similar results were obtained by concentrating and separating the MRSA isolates in blood sample. Recovery of the method was around 80 %.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table S1. Reproducibility of the migration times of the individual MRSA isolates belonging to the different genotype groups (A, B, and C). The separation conditions were the same as in the analysis shown in Figure 3.

Genotype	A			B			C		
Isolate	FS197	FS196	FS195	FS180	FS183	FS193	FS198	FS199	FS200
Run No.	Migration time [s]								
1	2207	2233	2231	2354	2334	2356	2272	2292	2315
2	2239	2230	2238	2321	2338	2341	2255	2269	2298
3	2242	2228	2259	2309	2341	2336	2262	2287	2309
4	2218	2217	2219	2322	2339	2338	2266	2303	2304
5	2244	2234	2225	2308	2358	2352	2304	2299	2273
6	2210	2241	2209	2340	2343	2355	2301	2306	2318
7	2231	2235	2225	2312	2335	2343	2275	2312	2316
8	2208	2236	2248	2318	2339	2361	2278	2279	2269
9	2223	2219	2251	2351	2350	2360	2281	2287	2287
10	2212	2216	2254	2323	2348	2356	2275	2313	2314
Mean	2223.4	2228.9	2235.9	2325.8	2342.5	2349.8	2276.9	2294.7	2300.3
value		2229.4			2339.4			2295.3	
SD [s]	13.84	8.28	15.85	16.76	7.47	8.90	15.58	14.46	18.14
RSD [%]	0.66	0.37	0.71	0.72	0.32	0.38	0.68	0.63	0.79

Figure captions

Figure 1. MEKC separation of MRSA genotype groups, A, B and C, and SEM images of the inner capillary surfaces; (A–D) optimization of the separation conditions, (E) detail view of the MEKC separation of the individual isolates of the genotype groups, A, B and C.

Figure 2. The effect of pH on the electroosmotic mobility in the smooth (curves 1 and 2) and SCW-etched FS capillary (curves 3–9) with respect to the BGE composition, rinsing procedure and temperature of the separation; neutral marker of EOF, thiourea; UV detection, 235 nm.

Figure 3. Separation of the individual MRSA isolates of the genotype groups A, B, and C under the optimized MEKC conditions. Detail view of the detected zones of the isolates.

Figure 4. Separation of MRSA isolates FS195, FS196 and FS197 (genotype group A) from spiked blood.

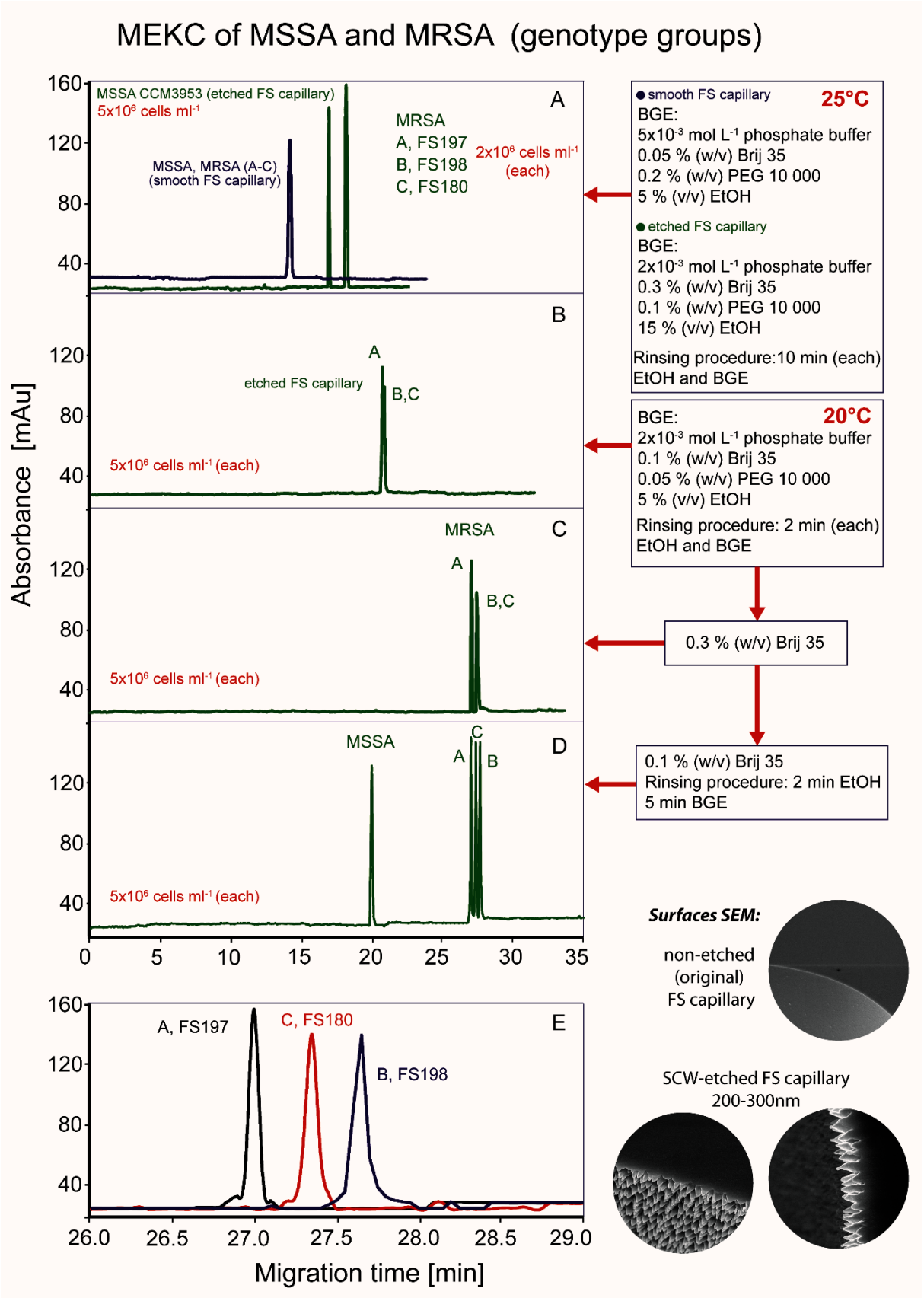
MEKC of MSSA and MRSA (genotype groups)
Absorbance [mAu]
MSSA CCM3953 (etched FS capillary)
5x10⁶ cells ml⁻¹
MSSA, MRSA (A-C)
(smooth FS capillary)
MRSA
A, FS197
B, FS198
C, FS180
2x10⁶ cells ml⁻¹
(each)
A
B
etched FS capillary
A
B,C
5x10⁶ cells ml⁻¹ (each)
C
MRSA
A
B,C
5x10⁶ cells ml⁻¹ (each)
D
MSSA
A
B
C
5x10⁶ cells ml⁻¹ (each)
E
A, FS197
C, FS180
B, FS198
Migration time [min]
smooth FS capillary
25°C
BGE:
5x10⁻³ mol L⁻¹ phosphate buffer
0.05 % (w/v) Brij 35
0.2 % (w/v) PEG 10 000
5 % (v/v) EtOH
etched FS capillary
BGE:
2x10⁻³ mol L⁻¹ phosphate buffer
0.3 % (w/v) Brij 35
0.1 % (w/v) PEG 10 000
15 % (v/v) EtOH
Rinsing procedure:10 min (each)
EtOH and BGE
BGE:
20°C
2x10⁻³ mol L⁻¹ phosphate buffer
0.1 % (w/v) Brij 35
0.05 % (w/v) PEG 10 000
5 % (v/v) EtOH
Rinsing procedure: 2 min (each)
EtOH and BGE
0.3 % (w/v) Brij 35
0.1 % (w/v) Brij 35
Rinsing procedure: 2 min EtOH
5 min BGE
Surfaces SEM:
non-etched
(original)
FS capillary
SCW-etched FS capillary
200-300nm

Figure 1

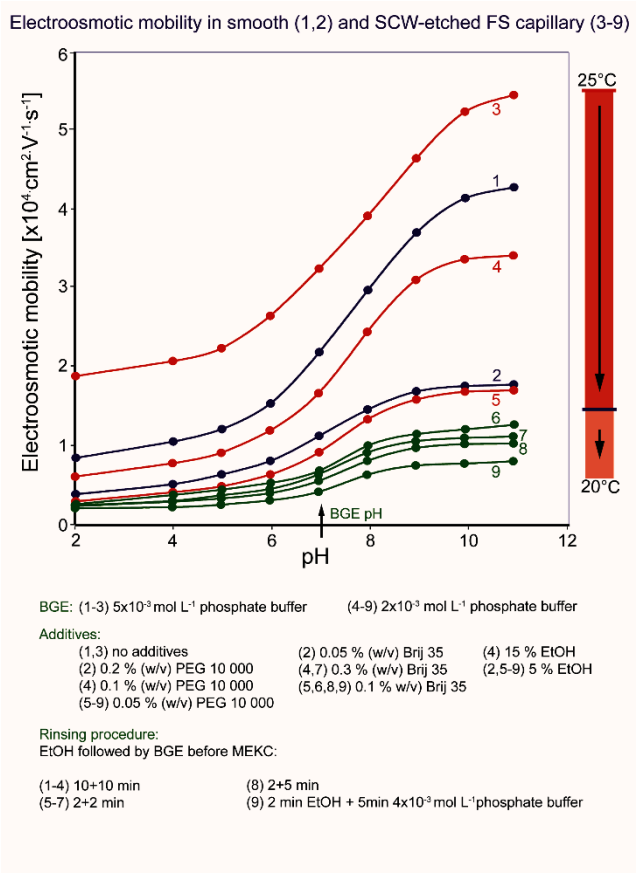

Figure 2

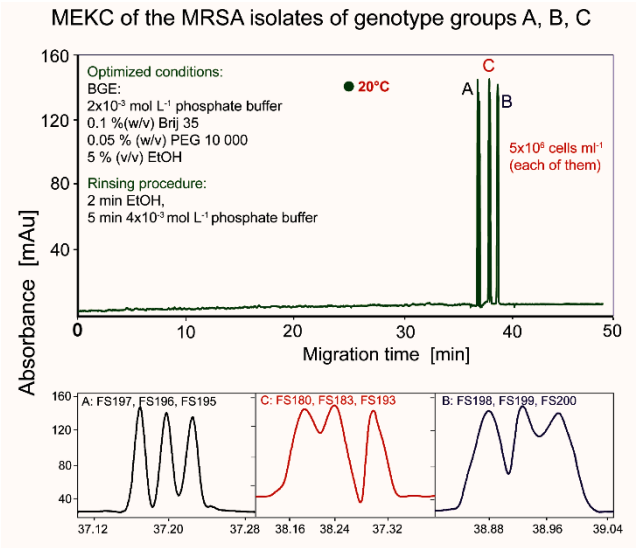

Figure 3

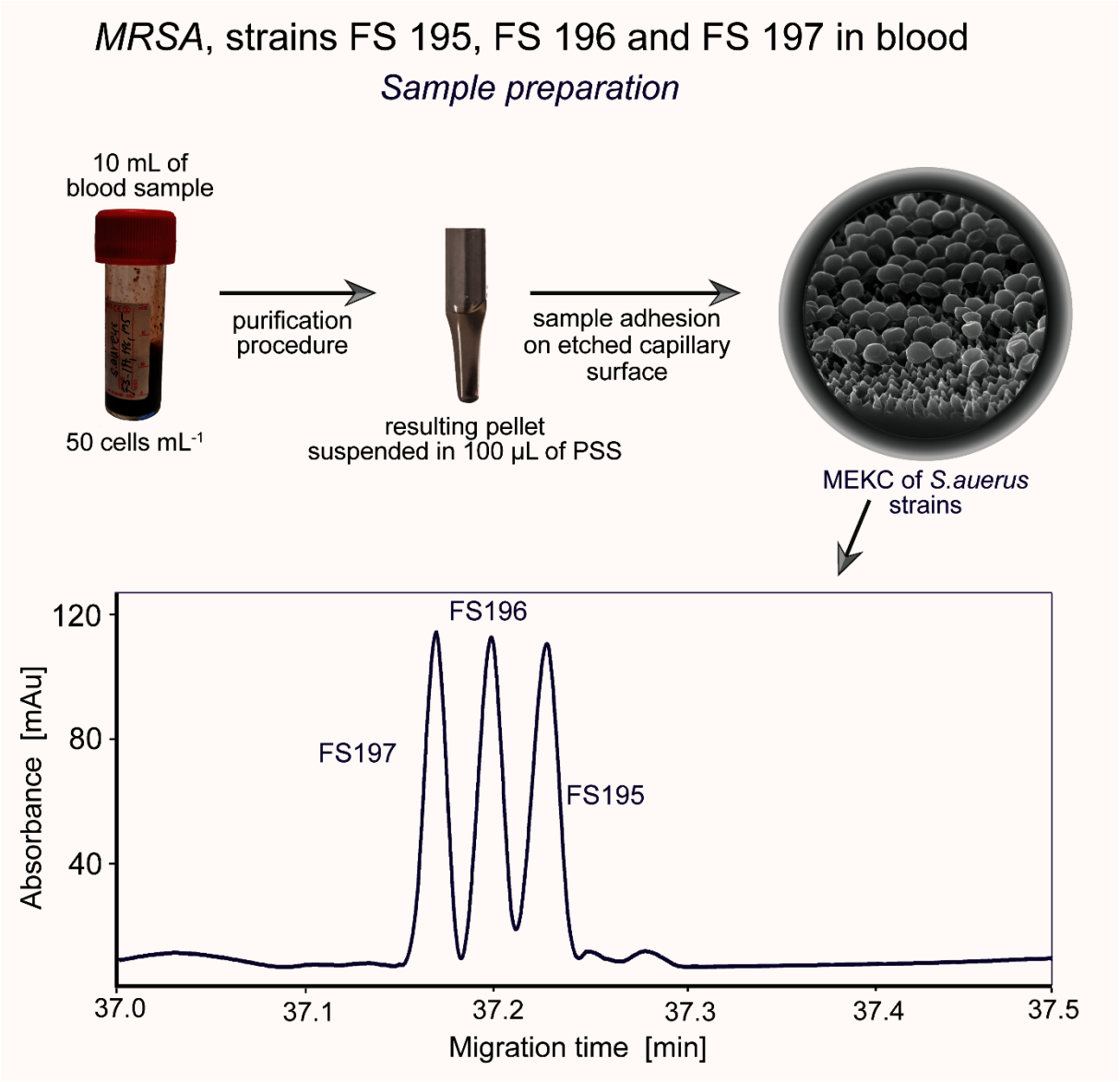
MRSA, strains FS 195, FS 196 and FS 197 in blood
Sample preparation
10 mL of blood sample
50 cells mL-1
purification procedure
resulting pellet suspended in 100 µL of PSS
sample adhesion on etched capillary surface
MEKC of S.auerus strains
FS196
FS197
FS195
120
80
40
Absorbance [mAu]
37.0
37.1
37.2
37.3
37.4
37.5
Migration time [min]

Figure 4