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Evaluation of *spa*-typing of methicillin-resistant *Staphylococcus aureus* using high-resolution melting analysis

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SUMMARY

Objective: *spa*-typing of methicillin-resistant *Staphylococcus aureus* (MRSA) has been used widely in clinical diagnostics and epidemiological studies. The aim of this study was to evaluate high-resolution melting (HRM) as a rapid and cost-effective method, to replace DNA-sequencing, for *spa*-typing in a global collection of 50 MRSA isolates.
Methods: The polymorphic X region of the *spa* gene was amplified by colony PCR using the SensiMix HRM kit, and the melting temperature (*Tm*) and melting curves of the amplicons were analyzed in close tubes using a Rotor-Gene 6000 instrument.
Results: Fifteen out of 19 *spa*-types each had a distinct *Tm*, which was sufficient to unambiguously type each of these *spa*-types. The remaining four *spa*-types could not be separated by *Tm* alone: t008 and t2770 shared a *Tm* (80.3 °C) and t021 and t311 shared a *Tm* (80.0 °C). However, they could be separated based on the shapes of their melting curves. There are discrepancies between the findings of the present study and those of previous studies, suggesting that standardization remains a challenge for cross-referencing.
Conclusion: HRM-based *spa*-typing is reproducible, simple, rapid, and cost-effective. t037 is prevalent in Brazil and Sudan, while diverse *spa*-types are found in Scotland and Saudi Arabia. Standardization is required for cross-referencing between laboratories globally.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major cause of infection in the community and healthcare settings, and poses a significant threat to public health.[1,2] Molecular typing methods are vital for the rapid identification of prevalent strains, which is important for active surveillance and to control the spread of disease. The polymorphic region of the gene encoding staphylococcal protein A (*spa*) has been found to be highly discriminatory and is useful for investigating both the local and global epidemiology of *S. aureus*.[3–9] The complexity and high running cost of PCR sequencing have limited its use in developing countries.[10] Recently, high-resolution melting (HRM)-based *spa*-typing has been described as a rapid and cost-effective method for genotyping locally predominant isolates.[10,11] To test the accuracy and reliability of HRM-based *spa*-typing, a global collection of 50 MRSA isolates was analyzed in the present study. HRM was demonstrated to accurately *spa*-type the majority of these isolates.

2. Materials and methods

2.1. MRSA isolates

Fifty clinical MRSA isolates collected between 2005 and 2012 were selected randomly from Scotland ($n = 22$), Brazil ($n = 13$), Sudan ($n = 3$), and Saudi Arabia ($n = 12$). All isolates were cultured and identified as *S. aureus*, as described previously.[12] The methicillin resistance phenotype was confirmed in accordance with the British Society for Antimicrobial Chemotherapy (BSAC) standards using the Vitek 2 system (bioMérieux, USA). An isolate was considered as methicillin-resistant when the minimum inhibitory concentration (MIC) breakpoint of oxacillin was >2 mg/l and cefoxitin >4 mg/l.[13]

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Table 1
HRM and *spa* sequence types of the 50 MRSA isolates, and the frequencies in the countries of origin

HRMT	*Tm*	*spa*-type	Repeat of *spa* type	Size, bp	CG%	Country (*spa* types/total, *n*/*n*)
1	79.4	t1544	07-22-34	72	44.4	Saudi Arabia (2/12)
2	79.5	t344	09-02-16-13-34	120	50	Scotland (5/22)
3	79.7	t044	07-23-12-34-34-33-34	168	41.7	Saudi Arabia (1/12)
4	79.8	t131	07-23-12-34-33-34	144	42.3	Saudi Arabia (1/12)
5	80.2	t304	11-10-21-17-34-24-34-22-25	216	43.5	Saudi Arabia (1/12)
6	**80.3**[a]	**t2770**	**07-23-12-12-21-17-34-34-33-34**	**240**	**42.5**	**Saudi Arabia (2/12)**
		t008	**11-19-12-21-17-34-24-34-22-25**	**240**	**43.7**	**Scotland (1/22)**
7	80.4	t138	08-16-02-25-17-24	144	45.1	Brazil (1/13)
8	80.6	t037	15-12-16-02-25-17-24	168	45.23	Brazil (12/13) and Sudan (3/3)
9	80.7	t363	15-16-02-25-17-24	144	45.8	Saudi Arabia (1/12)
10	80.8	t11986	04-44-33-31-31-12-34-16-12-25-22-34	285	43.5	Saudi Arabia (1/12)
11	80.9	t018	15-12-16-02-16-02-25-17-24-24-24	264	44.86	Scotland (6/19)
12	**81.0**[a]	**t311**	**26-23-17-34-20-17-12-17-16**	**216**	**44.9**	**Saudi Arabia (1/12)**
		t021	**15-12-16-02-16-02-25-17-24**	**216**	**44.9**	**Scotland (2/22)**
13	81.2	t002	26-23-17-34-17-20-17-12-17-16	240	45.4	Scotland (2/22)
14	81.3	t4573	07-23-13-23-31-05-17-25-16-28	240	45.0	Saudi Arabia (2/12)
15	81.4	t020	26-23-31-29-17-31-29-17-25-17-25-16-28	312	44.5	Scotland (1/22)
16	81.8	t4291	26-23-23-13-23-31-29-17-31-29-17-25-16-25-16-28	360	43.8	Scotland (1/22)
17	81.9	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	384	45.05	Scotland (4/22)

HRM, high-resolution melting; MRSA, methicillin-resistant *Staphylococcus aureus*; *Tm*, melting temperature.
[a] Note: *spa*-types in bold share the same *Tm*. These could be differentiated by the shapes of the melting curves, as shown in Figure 2.

2.2. DNA sequencing for spa-typing

The polymorphic regions of the *spa* gene were amplified and sequenced for all isolates, as described previously.[5,14] The sequence data were analyzed using the *spa*-typing plug-in in BioNumerics v. 5.1 (Applied Maths).

2.3. HRM analysis for spa-typing

The polymorphic X region of the *spa* gene was amplified in a Rotor-Gene 6000 instrument (Qiagen) by colony PCR using a SensiMix HRM kit (Bioline), as described previously by Shopsin et al.[3] In brief, a 20-μl PCR reaction was set up, containing 0.8 μl Eva-Green, 10 μl SensiMix, 1 μl of each primer (100 μM; 1095 forward 5′-AGACGATCCTTCGGTGAGC-3′ and 1517 reverse 5′-GCTTTTGCAATGTCATTTACTG-3′), and 20 ng of the template DNA; this was programmed as follows: a hold at 95 °C for 10 min, followed by 35 cycles of 95 °C for 20 s, 56 °C for 20 s, and 72 °C for 22 s. The high-resolution melting analysis of the amplicons was performed between 70 and 95 °C with a stepwise increase of 0.05 °C. The melting temperatures (*Tm*) were determined by the negative derivative of decreased fluorescence over increased temperature (*df*/*dt*), using the proprietary software (version 1.7.34). The shapes of the melting curves were viewed with the same software.

3. Results and discussion

By use of DNA sequencing and the *spa*-typing plug-in in BioNumerics, 19 *spa*-types were identified among the 50 isolates. *spa*-type t037 was the major *spa*-type that was prevalent among Brazilian (12/13 isolates) and Sudanese isolates (3/3 isolates). Scottish and Saudi Arabian isolates were quite diverse; eight *spa*-types were observed among 22 isolates from Scotland and nine *spa*-types among 12 isolates from Saudi Arabia (Table 1).

All 50 isolates were then subjected to HRM analysis. Fifteen out of the 19 *spa*-types each had a distinct *Tm* that unambiguously assigned 44 isolates (Table 1, Fig. 1). The melting curve within a *spa*-type was highly homogeneous. However, despite the difference in GC content between t008 and t2770 (43.7 mol% and 41.5 mol%, respectively), these *spa*-types shared the same *Tm* (80.3 °C; Fig. 2A). Similarly, t021 and t311 could not be separated from each other; they shared a *Tm* of 80.7 °C. This is probably due to the fact that they have the same GC content (44.9 mol%) (Fig. 2B).

Table 2
Comparison of melting temperatures (*Tm*) obtained in three independent studies

Tm	HRM-based *spa*-typing by:		
	Stephen et al., 2008[11]	Chen et al., 2013[10]	Present study, 2015
79.4			t1544
79.5			t344
79.6	t123		
79.7	t352, t065[a]		t044
79.8	t186		t131
80.0	t190		
80.1			
80.2			t304
80.3			t2770, t008[b,f]
80.4	t437[d]		t138
80.5			
80.6	t127, t008[a]		**t037**[e]
80.7	t019[d]		t363
80.8			t11986
80.9	**t037**[e], t1155[a]		t018[b]
81.0	t216		t311, t021[f]
81.1	t631		
81.2			**t002**[e]
81.3	t018[b]		t4573
81.4			t020
81.5			
81.6	**t002**[e]		
81.7			
81.8			t4291
81.9			t032[c]
82.0			
82.1		t9469	
82.2	t202		
82.3		t1081	
82.5		t9377	
82.6		t4677	
82.9		t701	
83.1		t437[d]	
83.2		t121	
83.3		t019[d]	
83.6		**t037**[e]	
84.0		t032[c]	
84.1		**t002**[e]	
84.3		t9970	

[a] *Tm* cannot distinguish *spa*-types in the study by Stephens et al.
[b] *Tm* discrepancy of *spa*-types between the present study and that by Stephens et al.
[c] *Tm* discrepancy of *spa*-type between the present study and that of Chen et al.
[d] *Tm* discrepancy of *spa*-types between the studies by Stephens et al. and Chen et al.
[e] *Tm* discrepancy in the three studies (text in bold).
[f] The melting curve shape could distinguish *spa*-types in the present study.

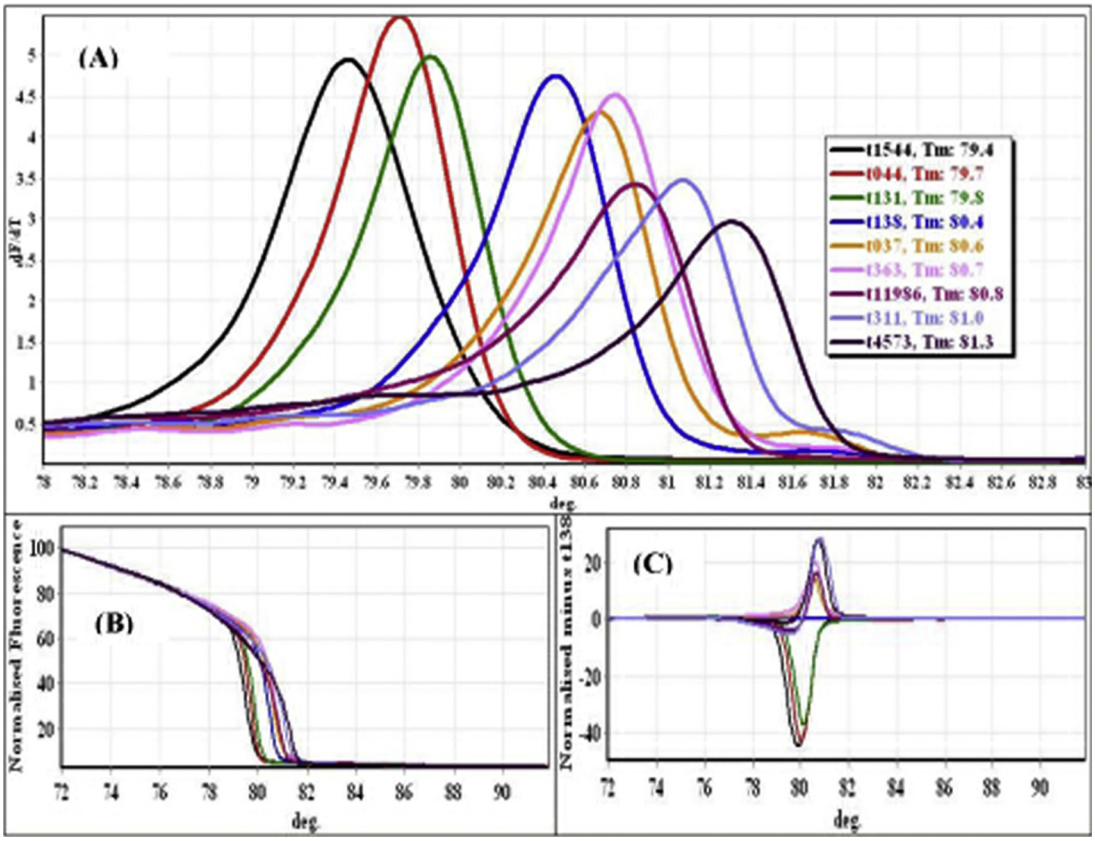

Figure 1. Comparison of different *spa* polymorphic region X HRM curves obtained from MRSA isolates. (A) Negative derivative of fluorescence over temperature (*df/dt*) plots displaying distinguishable HRM profiles. (B) Normalization data curve depicts the decreasing fluorescence vs. increasing temperature. (C) Difference graph demonstrating the accurate reproduction of eight *spa* HRM profiles in a run experiment.

These results are in agreement with those of Stephens et al.,[11] who found that two *spa*-types could not be separated from each other based on their *Tm*. It has been suggested that the shapes of the melting curves are also important in determining the *spa*-type,[11,15,16] and minor variations in the shapes of melting curves between t008 and t2770, and between t021 and t311 were also noticed in the present study (Fig. 2A and B). These variations in the shape of melting curves were reproducible; however inexperienced users might find this a bit complex for confidently predicting the correct *spa*-type. This highlights the challenge in optimizing HRM-based *spa*-typing for the growing number of *spa*-types of MRSA.

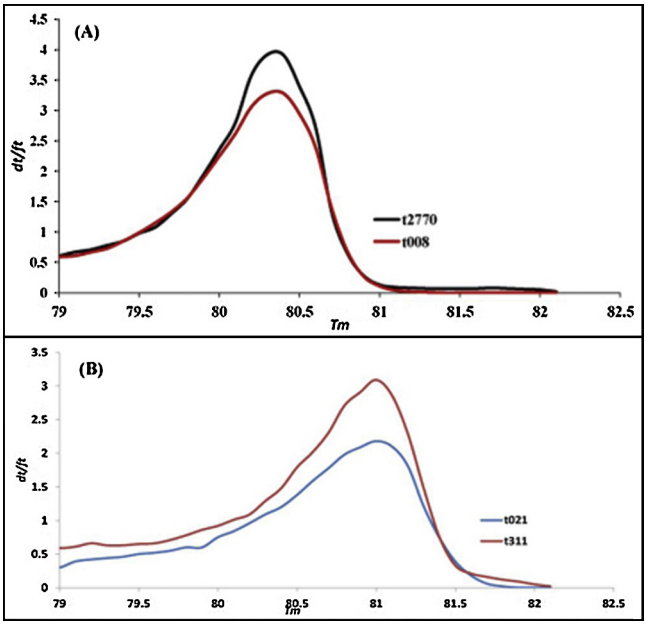

Figure 2. Melting curve shapes allowed the assignment of *spa*-types sharing the same *Tm*. (A) Characteristics of the melting curve shapes of t2770 and t008, respectively; they had identical *Tm* 80.3 °C. (B) Characteristics of the melting curve shapes of t021 and t311, respectively; they had identical *Tm* 81.0 °C.

Some discrepancies in the *Tm* values were also noted between this study and the previous investigations by Chen et al. and Stephens et al. (Table 2).[10,11] The *Tm* values for t037 were 80.9 °C, 83.6 °C, and 80.6 °C, and those for t002 were 81.6 °C, 84.1 °C, and 81.2 °C, in the Stephens et al. study,[11] Chen et al. study,[10] and the present study, respectively. Stephens et al. used Platinum SYBR-Green qPCR Super Mix-UDG (Life Technologies) on a Rotor-Gene 6000 instrument (Qiagen) and we used SensiMix HRM (Bioline) mix with Eva-Green dye on a Rotor-Gene 6000 instrument (Qiagen).[11] Chen et al. used LightCycler 480 HRM Master Mix containing ResoLight dye on a LightCyclerNano real-time PCR system (Roche).[10] The *Tm* values for both t037 and t002 were relatively closer between this study and that of Stephens et al., compared to the study of Chen et al., suggesting that the same instrument might provide similar *Tm* values for a *spa*-type and that minor variations may have been caused by different reaction mixes containing different reporting dyes. Further discrepancies in the *Tm* values between the studies of Stephens et al. and Chen et al. were observed for additional *spa*-types (Table 2). Therefore, different instruments and reagents (dye in the reaction mix) may result in discrepancies in the *Tm* values of a *spa*-type.

Taken together, it is concluded that HRM-based *spa*-typing is useful due to its reproducibility, simplicity, rapidity, and low cost. Standardization is needed for the laboratory screening of

Staphylococcus aureus spa-types globally. For the extension of its application to all *spa*-types and cross-referencing among laboratories worldwide, it is necessary to standardize and optimize the experimental conditions in each laboratory.

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References

1. Lucet JC, Chevret S, Durand-Zaleski I, Chastang C, Regnier B, Multicenter Study G.. Prevalence and risk factors for carriage of methicillin-resistant *Staphylococcus aureus* at admission to the intensive care unit: results of a multicenter study. *Arch Intern Med* 2003;**163**:181–8.
2. Matheson A, Christie P, Stari T, Kavanagh K, Gould IM, Masterton R, et al. Nasal swab screening for methicillin-resistant *Staphylococcus aureus*—how well does it perform? A cross-sectional study. *Infect Control Hosp Epidemiol* 2012;**33**: 803–8.
3. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999;**37**:3556–63.
4. Tang YW, Waddington MG, Smith DH, Manahan JM, Kohner PC, Highsmith LM, et al. Comparison of protein A gene sequencing with pulsed-field gel electrophoresis and epidemiologic data for molecular typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2000;**38**:1347–51.
5. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, 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**:5442–8.
6. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. *Spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol* 2004;**42**:792–9.
7. Malachowa N, Sabat A, Gniadkowski M, Krzyszton-Russjan J, Empel J, Miedzobrodzki J, et al. 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* 2005;**43**:3095–100.
8. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, the European Staphylococcal Reference Laboratory Working Group. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med* 2010;**7**: e1000215.
9. Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, et al., on behalf of the ESCMID Study Group of Epidemiological Markers (ESGEM). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Euro Surveill* 2013;**18**:20380.
10. Chen JH, Cheng VC, Chan JF, She KK, Yan MK, Yau MC, et al. The use of high-resolution melting analysis for rapid *spa* typing on methicillin-resistant *Staphylococcus aureus* clinical isolates. *J Microbiol Methods* 2013;**92**:99–102.
11. Stephens AJ, Inman-Bamber J, Giffard PM, Huygens F. High-resolution melting analysis of the spa repeat region of *Staphylococcus aureus*. *Clin Chem* 2008;**54**: 432–6.
12. Murray P, Jorgenson JH, Pfaller MAM, Yolken RH. Manual of clinical microbiology, 8th ed., ASM Press; 2003.
13. Andrews JM, Howe RA. BSAC standardized disc susceptibility testing method (version 10). *J Antimicrob Chemother* 2011;**66**:2726–57.
14. Sangal V, Girvan EK, Jadhav S, Lawes T, Robb A, Vali L, et al. Impacts of a long-term programme of active surveillance and chlorhexidine baths on the clinical and molecular epidemiology of meticillin-resistant *Staphylococcus aureus* (MRSA) in an intensive care unit in Scotland. *Int J Antimicrob Agents* 2012;**40**:323–31.
15. Ririe KM, Rasmussen RP, Wittwer CT. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* 1997;**245**: 154–60.
16. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chem* 2003;**49**:853–60.