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Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Switzerland: sampling only invasive isolates does not allow a representative description of the local diversity of clones

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Abstract

We conducted a molecular study of MRSA isolated in Swiss hospitals, including the first five consecutive isolates recovered from blood cultures and the first ten isolates recovered from other sites in newly identified carriers. Among 73 MRSA isolates, 44 different Double Locus Sequence Typing (DLST) types and 32 *spa* types were observed. Most isolates belonged to the NewYork/Japan, the UK-EMRSA-15, the South German and the Berlin clones. In a country with a low to moderate MRSA incidence, inclusion of non-invasive isolates allowed a more accurate description of the diversity.

Research note

According to the European Antimicrobial Resistance Surveillance Network (EARS-Net) data (www.ecdc.europa.eu), the proportion of invasive *Staphylococcus aureus* isolates that were found to be methicillin-resistant (MRSA) in European countries varied from below 5% to more than 50% in 2010. Switzerland is not included in the EARS-Net but has a national surveillance program for antibiotic resistance (www.anresis.ch). Overall the proportion of MRSA in clinical specimens was 9% in 2010 and 8% in 2011, varying from 4% in Central Switzerland to 14% in Western Switzerland.

Clonal lineages can be identified by molecular typing. Thus it is possible to follow the epidemiology and spread of major clones [1, 2]. A molecular epidemiological analysis conducted in 2006-2007 showed that MRSA *spa* types had a predominantly regional distribution in Europe [3]. Switzerland was not included in this study and no nation-wide molecular data were available since the last national study conducted in 1997 [4]. The objective of the present study was to assess the current molecular epidemiology of MRSA in Swiss hospitals.

Between January and June 2011, we conducted a survey of MRSA isolates collected in five > 500-bed Swiss hospitals (Basel, Bern, Lausanne, Luzern, and Zürich). Hospitals from the Italian-speaking region (Ticino) were also included and considered as one center. Participating hospitals had an incidence of new MRSA cases varying from below 1 to 18 per 1000 admissions (Table 1). Laboratories of participating centers were asked to collect up to five consecutive MRSA isolates from blood cultures in individual patients and the first ten consecutive MRSA isolates from clinical samples other than blood or from screening samples in newly identified MRSA carriers (one isolate per carrier). All isolates were sent to one reference center (Lausanne). Molecular analysis of MRSA strains was done by Double Locus Sequence Typing (DLST, a method based on the analysis of the highly variable regions of the *clf*B and *spa* genes) [5], *spa*-typing [6], and SCC*mec* typing [7]. The presence of Panton-Valentine leukocidin (PVL) genes was also investigated as described previously [8].

A total of 74 isolates were sent to the reference center: 14 isolates from blood cultures (zero to five per center) and 60 isolates from clinical or screening samples (ten per center). One isolate was found to be *mec*A negative (probable borderline oxacillin-resistant *S. aureus*; BORSA) and was excluded from the analysis. Depending on the hospitals' MRSA incidence, nine days to 3 months were needed to obtain isolates from ten newly identified patients.

Median age of patients was 63 years (range 1 to 99). Forty-eight (66%) were males. At the time of bacteraemia or first MRSA identification, 35 patients were hospitalized in wards, 7 in ICUs, 21 in emergency rooms or outpatient clinic; information was missing for 10 patients. Among the 14 patients with MRSA bacteraemia, 4 (29%) died within 14 days (all-cause mortality); ten episodes (71%) were hospital-onset bacteraemia.

Among the 73 MRSA isolates, 44 different DLST types (3 to 11 per hospital) and 32 *spa* types (2 to 9 per hospital) were observed (Table 1). Only 10 DLST types were shared by more

than one patient (2 to 12 patients). Nine different DLST types were found among the 14 MRSA strains isolated from blood cultures, whereas 38 different DLST types were found in the 59 other isolates.

DLST types were compared to typing data of international clones [7, 8]. At least 62 out of 74 (84%) MRSA isolates were related to 11 international clones (Table 2). Most of them belonged to the New York/Japan (ST5/105-SCC*mec* II- PVL neg; n=16), the UK-EMRSA-15 (ST22-IV-neg; n=15), the South German (ST228-I-neg; n=14) and the Berlin (ST45-IV-neg; n=4) clones. These data are consistent with the major international clones recovered in Europe [2]. However, the major clones currently observed in Swiss hospitals were different than those observed in 1997, except for the Berlin clone [9]. This illustrates a change in the molecular epidemiology of MRSA in Switzerland.

The genetic diversity encountered within each center varied according to the local epidemiology of MRSA. In the hospital with the highest MRSA incidence, 12 out of 15 isolates had the same DLST type indicating a large clonal outbreak in this center. In contrast, hospitals with a lower incidence showed a higher genetic diversity, suggesting a non-clonal spread of MRSA in these centers.

In this study, MRSA isolates were collected in few eligible centers with a low to moderate MRSA incidence during a limited period of time. Thus, only few invasive isolates were analyzed. Inclusion of non-invasive isolates allowed more isolates to be collected and to obtain a more accurate description of the diversity. Moreover, consecutive isolates were collected and the diversity observed in a hospital facing a clonal MRSA outbreak (such as center 1) was probably not representative of the real diversity observed year-round.

This study suggests that a national surveillance program could be easily implemented. Molecular analysis of ten isolates per center per year recovered throughout the whole year

should reflect the diversity of locally circulating MRSA clones. Only isolates of newly diagnosed MRSA cases (carriers or infected patients) should be included to better describe the incidence of circulating clones at the present time. DLST method allows the simultaneous analysis of 96 isolates in three days for an estimated cost of 20 euros per isolate and is therefore well suited for that purpose.

In conclusion, this study represents a recent update on the genetic diversity of MRSA observed in Switzerland. At least 11 international clones were recovered from the six participating centers. The small number of invasive isolates recovered in centers with a low MRSA incidence would not have allowed an accurate description of the local diversity. Adding none-invasive isolates represented a good alternative in settings with low MRSA incidence.

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Center	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Admissions (n)*	36112	37958	31149	34630	38924	36454	215227
New MRSA cases/1000 admissions*	18.05	0.63	1.09	1.24	1.95	1.34	4.15
Bacteremia due to MRSA/1000 adm.*	1.27	0.11	0.06	0.06	0.18	0.03	0.29
MRSA isolates (n)	15	13	10	10	15	10	73
Blood cultures	5	3	0	1	5	0	14
Other**	10	10	10	9	10	10	59
DLST types (n)	3	9	10	10	11	9	44
spa types (n)	2	9	9	8	8	9	32

* in 2010

** including screening samples

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Table 2. International clones recovered in Switzerland.

International clone (ST, SCC*mec*, PVL)	**Centers**	**DLST types (n)**	***spa* types**
New York/Japan (ST5/105-II-neg)	1, 2, 3, 4, 5, 6	2-2(9)	t002
		2-80(3)	t003
		2-414(1)	t586
		586-2(1)	t002
		665-80(1)	t003
		666-80(1)	t003
UK EMRSA-15 (ST22-IV-neg)	1, 4, 5	32-346(2)	t8849
		32-211(2)	t515
		32-623(2)	t2231
		32-627(1)	t2440
		32-341(1)	t3130
		32-626(3)	t4640
		32-628(1)	t7478
		251-211(1)	t515
		663-211(1)	t515
		664-211(1)	t515
South German (ST228-I-neg)	1, 2, 6	4-4(12)	t041
		4-338(1)	t579
		4-618(1)	ND
Berlin (ST45-IV-neg)	2, 3, 4, 6	1-198(1)	t050
		121-198(1)	t050
		641-37(1)	t015
		389-11(1)	t065
European CA-MRSA (ST80-IV-pos)	4, 6	7-26 (2)	t044
		646-26 (1)	t044
WA-MRSA-2 (ST88-IV-pos)	2, 3	69-63 (1)	t786
		604-409 (1)	t692
		653-287 (1)	t690
Lyon (ST8-IV-neg)	3	658-3 (1)	t008
		100-3 (1)	t008
Brazilian (ST239-III*merc.*-neg)	5	660-30 (2)	t037
WA-MRSA-1 (ST1-IV-neg)	4	5-46 (1)	t127
Southwest pacific (ST30-IV-pos)	2	51-94 (1)	t318
Livestock associated (ST398-V-neg)	4	245-339 (1)	t034

ST = sequence type according to the multilocus sequence typing (MLST) scheme

n = number of isolates

ND = not determined