

The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: Results of a second structured survey

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Citation style for this article:

Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, Glasner C, Sabat AJ, Weist K, Heuer O, Friedrich AW, on behalf of the ESCMID Study Group on Molecular Epidemiological Markers and the European Staphylococcal Reference Laboratory Working Group. The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: Results of a second structured survey. *Euro Surveill.* 2014;19(49):pii=20987. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20987>

Article submitted on 19 February 2014 / published on 11 December 2014

Staphylococcus aureus is one of the most important human pathogens and meticillin-resistant *S. aureus* (MRSA) presents a major cause of healthcare- and community-acquired infections. This study investigated the spatial and temporal changes of *S. aureus* causing bacteraemia in Europe over a five-year interval and explored the possibility of integrating pathogen-based typing data with epidemiological and clinical information at a European level. Between January 2011 and July 2011, 350 laboratories serving 453 hospitals in 25 countries collected 3,753 isolates (meticillin-sensitive *S. aureus* (MSSA) and MRSA) from patients with *S. aureus* bloodstream infections. All isolates were sent to the national staphylococcal reference laboratories and characterised by quality-controlled *spa* typing. Data were uploaded to an interactive web-based mapping tool. A wide geographical distribution of *spa* types was found, with some prevalent in all European countries. MSSA was more diverse than MRSA. MRSA differed considerably between countries with major international clones expanding or receding when compared to a 2006 survey. We provide evidence that a network approach of decentralised typing and visualisation of aggregated data using an interactive mapping tool can provide important information on the dynamics of *S. aureus* populations such as early signalling of emerging strains, cross-border spread and importation by travel.

Introduction

Staphylococcus aureus is one of the major causes of bacterial infection in humans [1]. Infections occur in the community or in healthcare settings, predominantly following acquisition from mainly human sources. In

Europe, meticillin-resistant *S. aureus* (MRSA) are predominantly acquired in healthcare settings and represent a major challenge to the control of antibiotic resistance in hospitals. MRSA has therefore become the currency with which the success of infection control initiatives is measured at health systems level [2]. *S. aureus* can also acquire particular virulence traits and has been responsible for major outbreaks of toxin-mediated disease in the community [3]. At the same time, *S. aureus* evolves gradually by successive acquisition of syntenic changes of largely unaltered core genomes. It is therefore possible to describe transmission and the consecutive spread of bacteria by genetic characterisation of highly polymorphic sites within the core genes of all isolates [4]. The importance of *S. aureus* as a human pathogen, i.e. its potential to cause large-scale outbreaks in healthcare settings and in the community, and its predominantly clonal population structure, calls for a monitoring tool that scans the distribution and spread of clones of particular public health importance over larger temporal and spatial intervals through repeated surveys. Such a tool is suited to inform public health and infection control personnel of impending health threats.

We therefore continued with a Europe-wide initiative to explore and define any dynamic changes in the distribution and spread of clones of *S. aureus* in European hospitals five years after an initial survey was carried out in 2006 [5]. We also addressed a request from the European Centre for Disease Prevention and Control (ECDC) to explore the usefulness of integration of molecular typing data with epidemiological and clinical data at a European level.

Methods

spa typing

Molecular typing for epidemiological purposes utilises highly discriminatory genetic markers that characterise human pathogens allowing the identification of isolates that are distinct versus those that are closely related due to recent common ancestry. The *spa* locus of *S. aureus* codes for Protein A, a species-specific gene product known for its IgG binding capacity. This locus is highly polymorphic due to an internal variable region of short tandem repeats which vary not only in number but also due to nucleotide substitutions within individual repeat units [6]. DNA sequences of the *spa* gene therefore provide portable and biologically meaningful molecular typing data that have demonstrated their utility for macro- and micro-epidemiological purposes from surveillance through to outbreak investigations at various geographical levels [7,8].

Capacity building

During annually repeated workshops organised for technical personnel from European Staphylococcal Reference Laboratories (SRL), participants receive hands-on training in *spa* typing and data analysis according to a standard protocol using a purpose-designed software tool StaphType (Ridom GmbH, Würzburg, Germany) [8]. Proficiency testing was carried out by mailing each SRL five well-characterised *S. aureus* isolates and five sequence chromatograms (trace files) of known *spa* types as described previously [9,10]. All laboratories participating in the structured survey described here fulfilled quantifiable quality criteria which consisted of an unambiguous base-calling for all sequenced nucleotides for both forward and reverse sequencing runs of the test panel.

Structured survey

A protocol was agreed by all participating SRLs in June 2010. Using the same network of sentinel laboratories, this by and large followed the sampling frame deployed of the first structured survey carried out in 2006 [5]. Briefly, European SRLs were asked to approach sentinel hospital laboratories which already participated in the previous survey and which provide microbiological diagnostic services for a geo-demographically representative sample for their national patient population. Between January and July 2011, these laboratories were asked to submit the first five consecutive MSSA and MRSA isolates from individual patients with blood stream infection, from each hospital the laboratories served. If, due to low incidence, five MRSA isolates could not be obtained within these six months, laboratories were entitled to make up their quota of 10 isolates by submitting additional MSSA isolates. For small countries with only one laboratory, such as Cyprus and Malta, more than 10 samples were accepted within this sampling period. Isolates were dispatched by the participating laboratories to the SRLs and, whenever possible, accompanied by additional information, including sample number, date of

isolation, demographic details (such as age and sex), epidemiological context (hospital-acquired if disease onset was more than 48 hours after admission, or community-onset for other cases), antibiotic resistance to isoxazolympenicillin (i.e. oxacillin) or ceftiofuran. SRLs confirmed MRSA by *mecA* PCR or determination of minimum inhibitory concentration for oxacillin together with PBP2a agglutination. Discrepancies between genotype or agglutination assay and susceptibility test were scored as inconclusive phenotypes. Additional information could be uploaded to the database and web application if available. This consisted of all-cause mortality 14 days after isolation of the initial blood-stream isolate. All SRLs preserved the isolates in strain collections and performed *spa* typing according to the standard protocol, uploaded the sequence information and made this available by synchronisation with the central Ridom SpaServer (www.spaserver.ridom.de) curated by SeqNet.org at the University Medical Center Groningen, the Netherlands [10,11]. Currently there are more than 13,000 *spa*-types and 630 repeat units stored on the SpaServer.

Epidemiological and typing data were communicated in parallel to a central purpose-designed structured query language (SQL) database at the Netherlands' National Institute for Public Health and the Environment (RIVM). For each local laboratory, SRLs also provided the postal address and decimal Cartesian coordinates for automatic geolocation. All data were anonymised and collected in accordance with the European Parliament and Council decision for the epidemiological surveillance and control of communicable disease in the European community [12,13]. Ethical approval and informed consent were thus not required.

Data analysis and geographical illustration

All data were inspected for inconsistencies and analysed on a country-by-country basis and returned to SRLs for feedback, clarification of inconsistencies and final approval in July 2012. After final approval, data were analysed using Stata version 11.0 (College Station, Texas, USA) using Pearson chi-squared test and Fischer's exact test for proportions and Student t-test for continuous variables. Quantitative differences with the 2006 survey were reported as results. The index of diversity (ID) is an unbiased measure of the probability of drawing two different *spa* types given the distribution of *spa* types in the sample. The 95% confidence intervals (CIs) were calculated as described previously [14]. Multilocus sequence typing (MLST) sequence types were extrapolated from the *spa* type as per the Ridom SpaServer. Cartesian coordinates were used for geolocation and plotting on Google Maps using the geocoding facility at www.spatialepidemiology.net [15]. The web application SRL-Maps (<http://www.spatialepidemiology.net/SRL-Maps2>) was developed to interrogate the data based on mapping of laboratory locations.

FIGURE 1

Geographical location of laboratories that contributed to survey of meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* isolates, 2011



Results

Summary statistics

Between January and July 2011, laboratories from 25 European countries participated in this survey. These included 22 European Union (EU) Member States (Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Malta, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom), two European Economic Area (EEA) countries (Iceland and Norway) and Switzerland. For the United Kingdom, Scotland participated on its own behalf, whereas England, Northern Ireland and Wales all referred

isolates to the same reference centre in England. Two countries (Cyprus and Malta) took the deliberate decision to carry out the necessary investigations in partner (twinning) laboratories, as the sample volume would not otherwise have justified the necessary investment for sequencing equipment. Since they were also allowed to submit a larger quota, they submitted 34 and 20 isolates respectively.

Altogether, 350 laboratories (Figure 1) serving 453 hospitals submitted data for 3,753 *S. aureus* isolates from patients with bloodstream infections, isolated during the six-month investigation period. Table 1 gives a summary overview of the number of participating

TABLE 1

Summary overview of participating laboratories, hospitals, number of invasive isolates of meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* and *spa* types by country, 25 European countries, 2011

Country	Number of laboratories	Number of hospitals	Number of isolates	MSSA	MRSA ^a	Number of <i>spa</i> types MSSA	Number of <i>spa</i> types MRSA	Number not typable	Percentage non-typable
Belgium	17	17	133	76	57	53	25	3	2.2
Bulgaria	11	11	46	33	13	25	8	0	0.0
Cyprus	1	1	34	20	14	17	10	1	2.9
Czech Republic	20	20	144	95	49	63	9	0	0.0
Denmark	16	58	288	276	10	150	7	2	0.7
Finland	17	43	173	163	10	89	5	2	1.2
France	34	34	286	166	120	98	31	3	1.0
Germany	10	26	161	100	61	62	22	0	0.0
Greece	1	13	85	47	38	33	15	0	0
Hungary	13	13	125	74	51	39	13	1	0.8
Iceland	1	1	9	7	2	6	2	0	0.0
Ireland	21	27	193	117	76	68	21	13	6.7
Italy	23	23	200	103	97	61	36	0	0.0
Latvia	8	8	36	33	3	17	2	1	2.8
Malta	1	1	20	10	10	10	7	0	0
Netherlands	2	16	138	130	8	77	5	7	5.1
Norway	22	n.d.	84	80	4	61	3	0	0.0
Poland	49	52	391	312	79	136	25	0	0.0
Portugal	1	19	195	99	96	52	23	0	0.0
Romania	5	5	37	15	22	13	7	0	0.0
Slovenia	10	13	164	142	22	40	3	9	5.5
Spain	1	30	129	35	94	30	27	0	0.0
Sweden	23	n.d.	225	215	10	114	10	1	0.4
Switzerland	6	6	60	46	14	39	9	1	1.7
United Kingdom: England, Northern Ireland and Wales	16	16	164	108	56	63	25	7	4.3
United Kingdom: Scotland	21	n.d.	233	119	114	69	35	0	0.0
Total	350	453	3,753	2,621	1,130	720	228	51	1.4

MSSA: meticillin-sensitive *Staphylococcus aureus*; MRSA: meticillin-resistant *S. aureus*; N.d.: not determined.

^a Note that the number of MRSA does not reflect a prevalence or occurrence in particular countries as the protocol asked for submission of the first five isolates of each phenotype.

laboratories and hospitals, isolates and *spa* types submitted by country. The combined collection consisted of 2,621 (69%) MSSA and 1,130 (31%) MRSA. Two isolates had an inconclusive resistance phenotype. One was *spa*-type t127 and the other *spa* non-typable.

A total of 861 different *spa* types were discerned, of which 720 were MSSA and 228 MRSA. Of these, 87 *spa* types were shared between MSSA and MRSA. When compared with results obtained during 2006, the *spa* type per isolate ratio remained at 0.23, indicating a similar sample diversity in both surveys. There were 51 isolates (out of a total of 3,753; 1.4%) which were *spa* non-typable. Typability of isolates ranged from 93.3% to 100% depending on the country.

Bloodstream infections with *S. aureus* occurred at an older age (median 68 years, Table 2) and predominantly in men, which is in accordance with previous findings [5,16]. The proportion of isolates from men was higher among MRSA than MSSA bloodstream infections ($p=0.03$). The median age at infection with MRSA was three years older than for infection with MSSA. Compared with 2006 data, the age distribution for both MSSA and MRSA in 2011 had shifted slightly to older age groups ($p<0.001$).

In 2011, data on all-cause mortality 14 days after index blood culture were available for 65.5% of all cases. Overall all-cause mortality was 19.4% (477/2,458). There was a difference between MRSA and MSSA in

TABLE 2

Staphylococcus aureus isolated from patients with blood stream infections, 25 European countries, comparison of 2006 and 2011 data

	2011					2006					p values comparing 2011 with 2006 ^d		
	n ^a	MSSA	MRSA	Total/overall ^b	p value ^c	na	MSSA	MRSA	Total/overall ^b	p value ^c	MSSA	MRSA	overall
Frequency (%)	3,753	2,621 (69.9)	1,130 (30.1)	3,751 (100.%)	-	2,890	1,923 (66.5)	967 (33.5)	2,890 (100%)	-	0.004	0.004	-
Median age (IQR)	3,753	67 (52-78)	70 (57-80)	68 (54-79)	<0.001	2,836	63 (46-75)	69 (55-78)	66 (49-76)	<0.001	<0.001	0.190	<0.001
Male sex (%)	3,702	1,572 (60.9)	723 (64.7)	2,295 (62.0)	0.029	2,862	1,159 (60.8)	606 (63.3)	1,765 (61.7)	0.2	0.994	0.504	0.768
All-cause mortality after 14 days (%)	2,458	289 (17.1)	188 (24.4)	477 (19.4)	<0.001	1,838	153 (13.2)	141(20.8)	294 (16.0)	<0.001	0.004	0.107	0.004
Hospital acquisition (%)	2,863	831 (44.0)	649 (66.6)	1,480 (51.7)	<0.001	2,322	777 (51.6)	585 (71.7)	1,362 (58.7)	<0.001	<0.001	0.020	<0.001
Number of spa types ^e	3,702	720	228	862 ^f	-	2,850	565	155	660 ^f	-	-	-	-
Number not typable	3,753	41 (1.6)	9 (0.8)	51 (1.4)	0.060	2,890	27 (1.4)	13 (1.3)	40 (1.4)	0.9	0.660	0.220	0.930
Index of diversity (95% CI)	3,688	0.986 (0.983 - 0.987)	0.942 (0.933 - 0.947)	0.985 (0.982 - 0.984)	<0.05 ^g	2,850	0.985 (0.983 - 0.987)	0.940 (0.933 - 0.947)	0.983 (0.982 - 0.984)	<0.05 ^g	-	-	-

MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *S. aureus*; IQR: interquartile range; CI: confidence intervals.

^a Number of isolates for which information was available for each variable. In 2011, two isolates had undetermined MRSA status.

^b Total number of isolates with an MSSA/MRSA status and data from the considered variable.

^c p-value for the comparison of MSSA with MRSA.

^d p-value comparing each of the three variables from 2011 with its counterpart from 2006 (e.g. MSSA 2011 with MSSA 2006).

^e Number of typeable isolates: MSSA= 2,580 and MRSA=4,121.

^f Total number of spa types includes 85 spa types that contain both MSSA and MRSA in 2011 and 60 spa types in 2006.

^g Deduced from non-overlapping 95% confidence intervals.

TABLE 3

The 20 most frequent *spa* types and multilocus sequence typing types among meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* isolates collected in 25 European countries in 2011

MSSA						MRSA					
Rank	<i>spa</i> type	Multilocus sequence type ^a	Frequency	%	Cumulative %	Rank	<i>spa</i> type	Multilocus sequence type ^a	Frequency	%	Cumulative %
1	t091	ST7	138	5.3	5.3	1	t032	ST22	202	17.9	17.9
2	t084	ST15	124	4.7	10.0	2	t003	ST225	99	8.8	26.6
3	t002	ST5	121	4.6	14.6	3	t008	ST8	95	8.4	35.0
4	t015	ST45	98	3.7	18.4	4	t002	ST5	87	7.7	42.7
5	t008	ST8	97	3.7	22.1	5	t067	ST125	50	4.4	47.2
6	t012	ST30	90	3.4	25.5	6	t041	ST228	24	2.1	49.3
7	t127	ST1	83	3.2	28.7	7	t777	ST5	21	1.9	51.2
8	t021	ST30	50	1.9	30.6	8	t018	ST36	20	1.8	52.9
9	t065	ST45	38	1.4	32.1	9	t022	ST22	20	1.8	54.7
10	t026	ST45	34	1.3	33.4	10	t037	ST239	19	1.7	56.4
11	t005	ST22	33	1.3	34.6	11	t127	ST1	18	1.6	58.0
12	t230	ST45	32	1.2	35.9	12	t747	ST22	17	1.5	59.5
13	t216	ST59	28	1.1	36.9	13	t044	ST80	15	1.3	60.8
14	t056	ST101	27	1.0	38.0	14	t2357	ST22	15	1.3	62.1
15	t148	ST72	25	1.0	38.9	15	t024	ST8	14	1.2	63.4
16	t024	ST8	23	0.9	39.8	16	t740	ST45	12	1.1	64.4
17	t346	ST15	23	0.9	40.7	17	t515	ST22	12	1.1	65.5
18	t571	ST398	23	0.9	41.5	18	t6057	ST22	11	1.0	66.5
19	t701	ST8	23	0.9	42.4	19	t030	ST239	9	0.8	67.3
20	t189	ST188	21	0.8	43.2	20	t014	ST225	9	0.8	68.1
Other	-	-	1,489	56.8	100.0	other	-	-	361	31.9	100.0
Total			2,621	100		Total			1,130	100	

MLST: multilocus sequence typing; MSSA: meticillin-sensitive *Staphylococcus aureus*; MRSA: meticillin-resistant *S. aureus*; %: percentage.

^a Predicted from *spa* typing data.

terms of all-cause mortality: 17.1% of patients with MSSA infections died, compared with 24.4% of patients with MRSA ($p < 0.001$). This difference was also identified in 2006 and is explained by various confounders that put MRSA patients at a higher risk of dying than those with MSSA infections [17]. Overall, there were more patient deaths in 2011 compared with the 2006 survey (16%, $p = 0.004$). Although observed for both MSSA and MRSA infections, this trend was only significant for MRSA infection ($p = 0.004$). Whether this difference indicates an evolution towards more virulence or changes in host factors such as the increase in age is something that cannot be determined from this dataset.

Disease onset occurred in the community for 56% of MSSA and 33.4% of MRSA infections, indicating that MRSA remains predominantly hospital-acquired. But there was a significant increase in the proportion of cases with community onset compared with the previous survey (in 2006 48.4% of MSSA infections had community onset, $p < 0.001$; for MRSA in the same year it was 28.3%, $p = 0.02$). A comparison of the most

prevalent *spa* types among hospital-acquired MRSA (HA-MRSA) and community-onset isolates (CO-MRSA) revealed little difference (not shown). In the 2011 sample, the five top ranking *spa* types comprised 52% and 45% of all HA-MRSA and CO-MRSA respectively.

The high overall diversity ($ID = 0.985$) is indicative of the good discriminatory ability of *spa* typing but, as with the 2006 sample, there has been a significant difference between MSSA and MRSA as a result of the oligoclonal nature of MRSA spreading through European countries.

Overall distribution of *spa* types

For MSSA, the top 20 ranking *spa* types included 43.2% of all MSSA isolates (Table 3). Importantly, there was very little difference among the first 11 ranking *spa* types between the 2011 and 2006 datasets. Only changes in rank order were observed. Ranks 12 to 20 contained four new *spa* types in 2011 (Table 3).

For MRSA, the top 20 ranking MRSA *spa* types contained 68.1% of all MRSA isolates (73.4% in 2006). There were no differences in the top six *spa* types (albeit in relative ranking). to32/ST22 now comprises 17.9% of all MRSA sampled in 2011 (up from 14.5% in 2006, $p=0.036$, Figure 2, Table 3). Except for t515, all ST22 related *spa* types (to32, to22, t747, t2357, t6057) have significantly increased in frequency and this lineage made up 36% of the top 20 ranking isolates in 2011, whereas in 2006 this figure was still lower at 23%. Three *spa* types have significantly decreased compared to the 2006 collection. too8/ST8, mainly found in France, decreased from 12.4% to 8.4% ($p=0.003$). to41/ST228 decreased from 7.4% in 2006 to 2.1% in 2011 ($p<0.001$). Finally, international clone to30/ST239 decreased from 2.1% to 0.8% ($p=0.013$).

Discussion

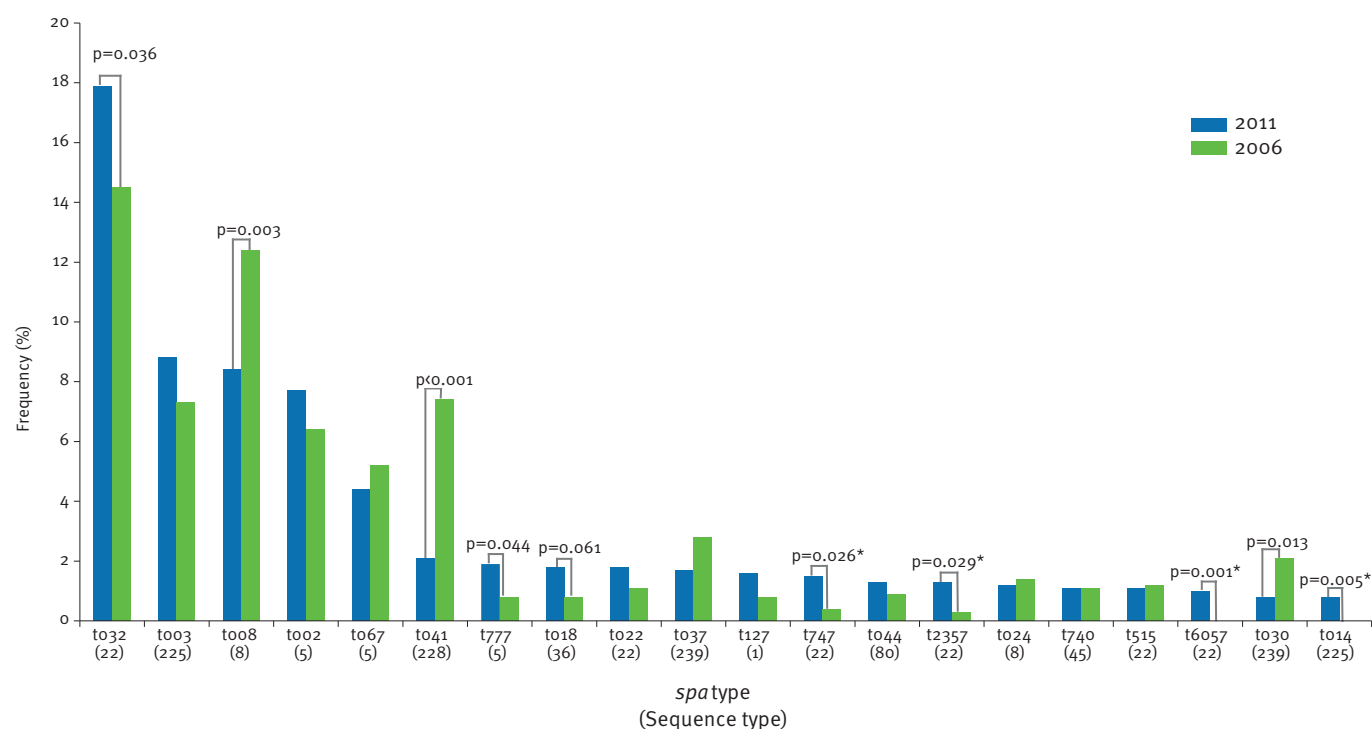
This survey represents a repetition of a previous study carried out in 2006 and was designed to investigate (i) the temporal and spatial changes of *S. aureus* clones of particular public health importance in Europe, and (ii) the feasibility and utility of integrating molecular typing data with epidemiological and clinical information at a European level.

We previously demonstrated the feasibility of creating a collaborative consortium of SRLs across Europe and alignment of *S. aureus* typing methodology in addition

to harmonising processes and data format at European level [5]. The continuation of this effort has shown that collaboration between countries can be maintained over extended intervals and provide added value to the understanding of the dynamic spread of *S. aureus* while quality, consistency of molecular typing and communication improves.

The results described here are testimony to the usefulness of structured surveys to generate information for public health action in a timely and economic fashion. Repeating surveys through previously created networks of sentinel hospital laboratories allows for consistent observations about the changing epidemiology of infections caused by bacterial clones of particular public health importance. In case of *S. aureus*, these clones are responsible for community and hospital-acquired infections, and they are often resistant to a range of antibiotic compounds and circulate among patients of extended hospital referral networks in Europe. They typically have a defined geographical distribution and show a steady diffusion along hospital patient referral lines. Moreover, our results suggest that HA-MRSA is filtering into the community at an increasing rate. The proportion of community-onset infections caused by international HA-MRSA clones has increased over the last five years from 28.3% to 33.4%. This difference is significant ($p<0.001$) and relevant as it indicates a trend to more export of hospital-associated clones

FIGURE 2
Comparison of meticillin-resistant *Staphylococcus aureus* *spa*-type frequencies, 2011 and 2006



Brackets over the bars indicate the bars for which p values were calculated.

into the community, probably as a result of patients' shorter hospital stays.

Among MRSA isolates, a dynamic expansion was demonstrated for several *spa* types. MRSA isolates with *spa* types belonging to ST22 increased most markedly making ST22 the most critically expanding MRSA clone in Europe. This lineage (designated EMRSA-15) was first described during hospital outbreaks in England. It caused a nationwide epidemic of healthcare-associated infections in the 1990s and is still the most prevalent HA-MRSA in the UK [18]. This clone has spread from the UK and Ireland and has become abundant in Germany, Hungary, Portugal and Northern Italy. MRSA belonging to *spa* type t018/ST36 has attained a foothold in Poland and t067/ST125, abundant in Spain during the 2006 survey [19], has been causing an outbreak in hospitals in a single health district in Finland in 2011 [20]. Among MSSA, *spa* type t571/ST398 appears to be spreading in France and Belgium [21]. Our observations indicate that infections with this clone are more frequent among younger men and may be associated with higher mortality. Its MRSA counterpart has been described as an ancestral human variant [22] of the livestock-associated MRSA clone ST398, which caused outbreaks of community-acquired infections in northern Manhattan that were linked to immigrants from the Dominican Republic [23]. Conversely, a reduction of international clone ST239 consisting of the *spa* types t030 and t037 and t041/ST228 was observed. It appears that the decline of ST239 is genuine as it mainly occurred in Poland, whereas the reduction of t041/ST228 can be explained by the fact that Austria and Croatia did not participate in the 2011 survey. Both countries contributed a high proportion of this type to the 2006 dataset. This highlights the importance of consistent participation in these types of pathogen-specific surveillance initiatives and the vulnerability of networks that depend on the goodwill and enthusiasm of participants.

Limitations of this study that deserve to be addressed include a deliberate decision that was taken by the SRLs to provide only isolates from bloodstream infections. This slight deviation from the sampling frame of the previous survey may have skewed the *spa* type distribution slightly. Moreover, the fixed number of isolates that were collected from each participating centre was due to the trade-off between the desire to make the workload of SRLs predictable and manageable and the inability to precisely determine incidence and the absolute increase or decrease of *spa* types. Thus, findings generated through these types of structured surveys must be put into context of surveillance data from other European-wide initiatives such as the European Respiratory Society's (ERS-net) and/or the Healthcare-Associated Infections Surveillance Network (HAI-net). The nature of structured surveys does not allow for early warning and response as it merely provides a rather static population snapshot of the *spa* types, i.e. clones that were extant and caused bloodstream

infections at the time of sampling. The value of these snapshots should not be underestimated, however, as they provide an unbiased view which can be used to identify clones of public health importance and their geographic abundance and can inform ad hoc epidemiological investigations about the dignity and geographical origin of organisms isolated during outbreaks.

The exchange of typing results using an illustrative mapping tool such as the spatialepidemiology.net website's SRL-maps provides the means to determine the reach and expansion of clones with proven success simultaneously for different countries. Initiatives such as these could lead to an improved and sustainable effort to control and eradicate emerging high-risk clones at the level of healthcare institutions once international agencies secure the sustainability for these repeated efforts.

A consistent integration of typing data with pre-existing epidemiological and or clinical data collected through other European surveillance initiatives (such as the European Antimicrobial Resistance Surveillance Network (EARS-net), European Surveillance of Antimicrobial Consumption Network (ESAC-net) or HAI-net) will, depend on the successful implementation of further alignment of sampling methodology, diagnostic procedures and of the regulatory framework across Europe. It would require a systematic and internationally accepted identification-code for hospitals and diagnostic laboratories, as well as for bacterial isolates, which are reported through different surveillance initiatives and for patients from whom these organisms were originally recovered. This would require novel regulatory approaches on the part of European national governments. Moreover, data protection and confidentiality issues would need to be resolved before such regulations could be enacted. The alternative would be a fully decentralised approach. This would require additional efforts from hospitals and laboratories to provide pertinent epidemiological and clinical information in addition to molecular typing and antibiotic susceptibility data and to report this bundled information while maintaining full confidentiality. However, as such efforts require a considerable degree of reorganisation they may seem unrealistic under the current climate of austerity. During the deliberations with the representatives of the SRLs in our study, the possibility of collecting more accurate data about source patients (epidemiology and clinical outcome) was appraised. The prevailing consensus was that this would be unrealistic given the scarcity of information provided to diagnostic laboratories by clinicians on the request forms and the inability of laboratories to fund these additional enquiries from their own budgets. It is important to note that these concerns were not raised by single members of the SRL working group, but appear to represent a common view.

In conclusion, collaborative typing initiatives are able to identify the continental spread of high-risk clones

across national boundaries and can indicate to health-care providers the emergence of threats caused by successful and antibiotic-resistant bacteria. The geographic diffusion of antibiotic-susceptible and resistant clones of *S. aureus* can be made visible with the help of intuitive information tools such as interactive websites. This can improve the coherence of individual laboratory results and contribute to a better understanding of the population dynamic of these important pathogens. A simple integration of typing data with data from other existing surveillance efforts is, however, currently constrained by regulatory hurdles and legitimate concerns about patient data protection.

Members of the European Staphylococcal Reference Laboratory Working Group

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Acknowledgements

The authors would like to like to express their gratitude to all participating laboratories and hospitals throughout the European region for sharing their isolates and demographic data for the study. We would also like to thank Roel Coutinho, Director of the Center for Infection Control at RIVM for his encouragement and supporting the organisational effort of this initiative and Carola Schinkel of RIVM and Tomorrow's Events for the organisation of the annual training and planning workshops. The support and collaboration with the ESCMID Study Group of Epidemiological Markers (ESGEM) is also gratefully acknowledged. The study was funded by ECDC through tender and framework contract ECDC 09/033.

Conflict of interest

None declared.

Authors' contributions

ICMJE criteria for authorship read and met: HG AWF. All authors agreed with the manuscript's results and conclusions. HG, AWF and the European Staphylococcal Reference Laboratory Working Group designed the experiments and the study. HG and AT analysed the data. LS, GP, MC, CG, AS and HG collected data and/or did experiments for the study. HG wrote the first draft of the paper. KW and OH contributed to the writing of the paper. DMA developed the public domain Web-based interactive mapping tool.

References

- Lowy FD. Staphylococcus aureus infections. *N Engl J Med.* 1998;339:520-32. <http://dx.doi.org/10.1056/NEJM199808203390806>
- Harbarth S, Pittet D. MRSA--a European currency of infection control. *QJM.* 1998;91(8):519-21. <http://dx.doi.org/10.1093/qjmed/91.8.519>
- McAdam PR, Templeton KE, Edwards GF, Holden MT, Feil EJ, Aanensen DM, et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant Staphylococcus aureus. *Proc Natl Acad Sci U S A.* 2012;109(23):9107-12. Epub 2012 May 14. <http://dx.doi.org/10.1073/pnas.1202869109>
- Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC et al. (2003) How clonal is Staphylococcus aureus? *J Bacteriol.* 2003;185:3307-16. <http://dx.doi.org/10.1128/JB.185.11.3307-3316.2003>
- Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW; European Staphylococcal Reference Laboratory Working Group. Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med.* 2010;7(1):e1000215. <http://dx.doi.org/10.1371/journal.pmed.1000215>
- Frénay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, et al. Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. *Eur J Clin Microbiol Infect Dis.* 1996; 15:60-4. <http://dx.doi.org/10.1007/BF01586186>
- Harmsen D, Claus H, Witte W, Rothgänger 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. <http://dx.doi.org/10.1128/JCM.41.12.5442-5448.2003>
- Mellmann A, Friedrich AW, Rosenkötter N, Rothgänger J, Karch H, Reintjes R, et al. Automated DNA sequence-based early warning system for the detection of methicillin-resistant Staphylococcus aureus outbreaks. *PLoS Med.* 2006;3:e33. <http://dx.doi.org/10.1371/journal.pmed.0030033>
- Aires-de-Sousa M, Boye K, de Lencastre H, Deplano A, Enright MC, Etienne J, et al. High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. *J Clin Microbiol.* 2006;44:619-21. <http://dx.doi.org/10.1128/JCM.44.2.619-621.2006>
- Friedrich AW, Witte W, Harmsen D, de Lencastre H, Hryniewicz W, Scheres J, et al. SeqNet.org: a European laboratory network for sequence-based typing of microbial pathogens. *Euro Surveill.* 2006 Jan 12;11(1):E060112.4.
- Friedrich AW, Mellman A, Harmsen D. Spa sequence typing home page. [Accessed 28 September 2009]. Available from: <http://www.seqnet.org/>
- The European Parliament and the Council of the European Union. Decision number 2119/98/EC of the European Parliament and of the Council of 24 September 1998: setting up a network for the epidemiological surveillance and control of communicable diseases in the community. *Official Journal of the European Union.* Luxembourg: Publications Office of the European Union. 03.10.1998: L 268. Available from: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:268:0001:0006:EN:PDF>
- European Commission. Commission Decision of 22 December 1999 on the communicable diseases to be progressively covered by the Community network under Decision No 2119/98/EC of the European Parliament and of the Council (notified under document number C(1999) 4015) (2000/96/EC). EC; 2000. *Official Journal of the European Union.* Available from: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000D0096:20120905:EN:PDF>
- Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol.* 2001;39:4190-2. <http://dx.doi.org/10.1128/JCM.39.11.4190-4192.2001>
- Aanensen DM, Spratt BG. *Spatialepidemiology.net*. Web mapping application for Infectious Disease Epidemiology. [Accessed 28 September 2009.] Available from: <http://www.spatialepidemiology.net>.
- Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis.* 2005;5(12):751-62. [http://dx.doi.org/10.1016/S1473-3099\(05\)70295-4](http://dx.doi.org/10.1016/S1473-3099(05)70295-4)
- de Kraker ME, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, et al. Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus

- bloodstream infections. *Antimicrob Agents Chemother.* 2011;55(4):1598-605. <http://dx.doi.org/10.1128/AAC.01157-10>
18. Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, Harris SR, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res.* 2013;23(4):653-64. <http://dx.doi.org/10.1101/gr.147710.112>
 19. Pérez-Vázquez M, Vindel A, Marcos C, Oteo J, Cuevas O, Trincado P, et al. Spread of invasive Spanish *Staphylococcus aureus* spa-type to67 associated with a high prevalence of the aminoglycoside-modifying enzyme gene *ant(4')-Ia* and the efflux pump genes *msrA/msrB*. *J Antimicrob Chemother.* 2009;63(1):21-31. <http://dx.doi.org/10.1093/jac/dkn430>
 20. Laine J, Huttunen R, Vuento R, Arvola P, Levola R, Vuorihuhta M, et al. Methicillin-resistant *Staphylococcus aureus* epidemic restricted to one health district in Finland: a population-based descriptive study in Pirkanmaa, Finland, years 2001-2011. *Scand J Infect Dis.* 2013;45(1):45-53. <http://dx.doi.org/10.3109/00365548.2012.710853>
 21. Vandendriessche S, Kadlec K, Schwarz S, Denis O. Methicillin-susceptible *Staphylococcus aureus* ST398-t571 harbouring the macrolide-lincosamide-streptogramin B resistance gene *erm(T)* in Belgian hospitals. *J Antimicrob Chemother.* 2011;66(11):2455-9. <http://dx.doi.org/10.1093/jac/dkr348>
 22. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio.* 2012;3(1). pii: e00305-11. <http://dx.doi.org/10.1128/mBio.00305-11>
 23. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, Kennedy AD, et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *MBio.* 2012;3(2). pii: e00027-12. <http://dx.doi.org/10.1128/mBio.00027-12>