

Perspectives

A EUROPEAN LABORATORY NETWORK FOR SEQUENCE-BASED TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AS A COMMUNICATION PLATFORM BETWEEN HUMAN AND VETERINARY MEDICINE – AN UPDATE ON SEQNET.ORG

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8. 44 participating European laboratories (listed in Table 1)

Introduction of SeqNet.org

SeqNet.org is currently an initiative of 44 laboratories from 25 European countries and one laboratory from Lebanon (Table 1), founded in 2004, in collaboration with the Robert Koch Institute at the University of Münster, Germany (<http://www.SeqNet.org>). Since then, its main objective is to establish a European network of excellence for sequence-based typing of microbial pathogens, having its main focus on *Staphylococcus aureus* [1]. SeqNet.org comprises a large number of national reference laboratories as well as university laboratories. The principle goal of SeqNet.org is to generate unambiguous, easily comparable typing data in electronic, portable form to be used by infection control at a local level as well as national and European surveillance of sentinel micro-organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA). *spa*-typing has been shown to be a useful tool in molecular hospital epidemiology [2,3]. Veterinary laboratories have recently joined the SeqNet.org initiative as MRSA has become an emerging problem in veterinary medicine [4,5]. *spa*-typing data from human and veterinary medicine can be compared using the *spa* server database [6].

SeqNet.org objectives

1. Organisation and participation in seven international workshops contributed to the harmonisation of sequencing methods for sequence-based typing of MRSA and the capacity for building DNA sequencing in diagnostic microbiology. Further meetings and workshops are planned.
2. SeqNet.org rules require that SeqNet.org laboratories (Table 1) undergo at least one certification trial [7] for sequence-based typing of MRSA. Regular proficiency tests are foreseen.
3. Curatorship of the Ridom *spa* server and the development and maintenance of a SeqNet.org web-portal allows the transfer of data at an international level.
4. The excellence of data quality needs to be maintained. This is necessary as the access to the *spa* server will be enlarged in the future.

SeqNet.org database

SeqNet.org is co-ordinated by the University Hospital in Münster and the Robert Koch Institute in Wernigerode, Germany. Besides ensuring the quality aspect, SeqNet.org is responsible for curating the *spa* server for all laboratories using the *spa* server. Currently, the 44 SeqNet.org participating European laboratories (Table 1, Figure) and 148 other laboratories submitting data have synchronised more than 3,816 *spa* types consisting of 222 *spa* repeats from 59,401 *S. aureus* strains of which 93% were MRSA. The analysis of more than 27,000 *spa* server submissions show that the 30 most frequent *spa* types cover 66% of all submissions (Table 2).

FIGURE

Proportion of strain submissions with complete data set to the *spa* server, by country in Europe, SeqNet.org curated Ridom *spa* server, 1 April 2004 – 15 February 2008 (n = 32,544)

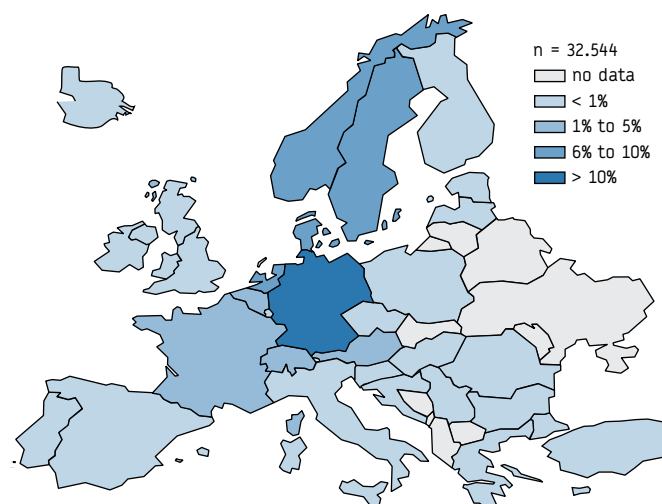


TABLE 1

SeqNet.org participating medical and veterinary laboratories and institutions (44 in Europe, one in Lebanon)

Nr	Organisation	Veterinary (V) Medical (M)	Main contact person	City	Country
1	Institut für Hygiene, Mikrobiologie und Tropenmedizin	M	H. Mittermayer	Linz	Austria
2	Österreichische Agentur für Gesundheit und Ernährungssicherheit	V	F. Allerberger	Wien	Austria
3	ULB-Hopital Erasme-National Reference Laboratory for Staphylococci	M	M. Struelens	Bruxelles	Belgium
4	National Center of Infectious and Parasitic Diseases	M	T. Kantardjiev, D. Nashev	Sofia	Bulgaria
5	National Institute of Public Health	M	H. Zemlicková	Prague	Czech Republic
6	Clinical and Molecular Microbiology, Clinical Hospital Centre Zagreb	M	S. Kalenic A. Budimir	Zagreb	Croatia
7	Hvidovre Hospital	M	H. Westh (Advisory Board)	Hvidovre	Denmark
8	Statens Seruminstitut	M	R. Skov	Copenhagen	Denmark
9	National Food Institute (DTU)	V	H. Hasman	Copenhagen	Denmark
10	National Public Health Institute	M	J. Varkkila	Helsinki	Finland
11	Centre National de Référence des Staphylocoques	M	J. Etienne H. Meugnier	Lyon	France
12	Institute of Hygiene (1), University Hospital Muenster, Clinic for Periodontology (2), University Hospital Münster	M	A. W. Friedrich (1) (Co-ordinator) A. Mellmann (1), D. Harmsen (2)	Muenster	Germany
13	Institute of Medical Microbiology, University Hospital Muenster	M	G. Peters, K. Becker	Muenster	Germany
14	Institute of Hygiene and Microbiology, University of Wuerzburg	M	U. Vogel	Wuerzburg	Germany
15	Robert Koch Institute	M	W. Witte (Co-ordinator)	Wernigerode	Germany
16	Charité – University Medicine Berlin	M	K. Weist	Berlin	Germany
17	Institute of Medical Microbiology and Hospital Hygiene, University Hospital Düsseldorf	M	R. Schulze-Röbbecke	Düsseldorf	Germany
18	Institut für Medizinische Mikrobiologie und Hygiene, University Hospital Tübingen	M	V. Kempf, B. Schulte	Tübingen	Germany
19	University of Athens	M	A. Tsakris E. Piperaki	Athens	Greece
20	“Johan Bela” National Center for Epidemiology	M	M. Fuzi	Budapest	Hungary
21	Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental School & Hospital	M	A. Shore	Dublin	Ireland
22	National MRSA Reference Laboratory, St James’s Hospital, Dublin	M	A. Rossney	Dublin	Ireland
23	Istituto Superiore di Sanità, National Reference Laboratory on Antimicrobial Resistance	M	A. Pantosti, M. Monaco	Rome	Italy
24	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, National Reference Laboratory on Antimicrobial Resistance	V	A. Battisti, R. Lorenzetti	Rome	Italy
25	P. Stradins Clinical University Hospital	M	E. Miklasevicz	Riga	Latvia
26	Lebanese American University, Microbiology & Biotechnology	M	S. Tokajian	Byblos	Lebanon
27	Laboratoire National de Santé, Microbiology unit	M	J. Mossong	Luxembourg	Luxembourg
28	Laboratorium Microbiologie Twente Achterhoek	M	R. Hendrix	Enschede	The Netherlands
29	National Institute of Public Health (RIVM)	M	X. Huijsdens	Bilthoven	The Netherlands
30	University Hospital	M	E. Stobberingh	Maastricht	The Netherlands
31	St. Olavs University Hospital, National Reference Laboratory	M	T. Jacobson	Trondheim	Norway
32	Akershus University Hospital	M	T. Taennes	Lørenskog	Norway
33	Telelab	M	Y. Tveten	Skien	Norway
34	National Medicines Institute	M	W. Hryniewicz (Advisory Board)	Warsaw	Poland
35	Instituto de Tecnologia Química e Biológica (ITQB)	M	H. de Lencastre (Advisory Board)	Oeiras	Portugal
36	National Institute for Research and Development for Microbiology and Immunology	M	I. Codita	Bucharest	Romania
37	Microbiology Department, Stobhill Hospital	M	D. Morrison E. Giles	Glasgow	Scotland
38	University of Ljubljana/Medical Faculty	M	M. Mueller-Premru	Ljubljana	Slovenia
39	Centro Nacional de Microbiología (Instituto de Salud Carlos III)	M	J. Campos	Madrid	Spain
40	Lund University Hospital	M	A.-C. Petersson	Lund	Sweden
41	Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet)	M	S. Haeggman	Solna	Sweden
42	Sahlgrenska University Hospital, Göteborg	M	Ch. Welinder-Olsson	Goteborg	Sweden
43	Universitätsspital Basel, Mikrobiologie	M	R. Frei	Basel	Switzerland
44	Staphylococcus Reference Laboratory, Health Protection Agency	M	A. Kearns	London	UK
45	Health Protection Agency, Laboratory of Healthcare Associated Infection and HARMONY IUMS co-ordinator	M	B. Cookson	London	UK

Since 2006, the analysis with the BURP (Based Upon Repeat Pattern) algorithm makes it possible to group the *spa* types by means of their relatedness to each other and to a common founder [8,9]. Occasionally, misclassifications between Multilocus Sequence Typing (MLST) and *spa* occur due to intergenomic recombination [10,11] or large chromosomal replacement comprising the *spa* locus, leading to outliers, such as described for ST239 and ST34 [12]. However, BURP analysis shows a correspondence of 92% for pulsed-field gel electrophoresis (PFGE) patterns and 97% for MLST clonal clusters, so that *spa*-typing generated within the SeqNet.org network are comparable with PFGE and MLST databases [10,11]. For example, MRSA strains belonging to MLST type ST398 have been recently associated with pigs. They correspond to the *spa* Clonal Complex t011 (*spa* types t011, t034, t108) and these *spa* types can be used as identifying markers for such strains isolated from humans and animals [5]. The *spa* database is, in its current form, essentially used as a dictionary assuring a common nomenclature, providing molecular typing data in real time, and maintaining excellence of typing data quality. Its data on frequencies of *spa* types can already at this stage provide valuable information regarding wider geographical dissemination (Table 1, Figure). In interpreting raw *spa* data from the *spa* database the following aspects need to be considered:

- Different sampling schemes in different countries. Specifically, a few types might be overrepresented because of focussing on special topics such as: 1) pig farming and MRSA ST398 in Germany, the Netherlands, and Belgium; 2) Panton-Valentine leukocidin-positive (PVL-positive) t044 (ST80) looking especially

for presumptive community-acquired CA-MRSA of the European clone; and 3) t084 due to a detailed study on dissemination of MRSA ST8/ *spa* t084 in Denmark. Furthermore, the same *spa* type can designate MRSA and MSSA (methicillin-sensitive *S. aureus*) isolates. Therefore, resistance must always be confirmed before.

- Geographical dissemination. Reporting particular types from many countries is an indicator for epidemic spreading, but it does not necessarily indicate wide geographical dissemination of a special clone. Here, a convergent evolution from a frequent MSSA ancestor is also possible, as already indicated by the possession of different types of SCCmec elements as in t002 (ST5) and t008 (ST8).
- Confirmation with additional testing. Identification of CA-MRSA ST8 ("USA300" clone) is likely when the isolates originate from deep infections of skin and soft tissue [13]. A confirmation of CA-MRSA ST8 should be performed by using PCR for *lukS*-PV *lukF*-PV and *arC*. Therefore, *spa*-typing can be useful as surrogate marker for highly epidemic and highly prevalent clones [14], but further microbiological characterisation is necessary [11].

Recent developments

In April 2007, the SeqNet.org plenary meeting was held in Rhodes, Greece and was aimed to exchange experiences, discuss and decide on questions which arose during the last three years of sequence-based typing throughout Europe. SeqNet.org participating laboratories presented their experiences in using *spa*-typing as

TABLE 2

Ten most frequently synchronised *spa* types on the SeqNet.org curated Ridom *spa* server, 59,401 submissions, 1 April 2004 - 6 May 2008

<i>spa</i> -type	Frequency	Countries of origin	<i>spa</i> -CC	MLST	Comment and other designations
t003	12.64 %	Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Netherlands, Norway, Sweden, Switzerland, United States	CC001	ST-5, ST-225	CC5, Rhine Hesse MRSA (subclone), EMRSA-3, New York clone
t032	9.78 %	Austria, Belgium, Czech Republic, Denmark, France, Germany, Iceland, Italy, Lebanon, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom, United States	CC032	ST-22	Barnim MRSA (prototype & subclone), EMRSA-15*, prototype of ST-22, CC22
t008	6.94 %	Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Iceland, India, Israel, Italy, Japan, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom, United States	CC024	ST-8, ST-247, ST-250, ST-254	CC8, Northern German MRSA (subclone), USA300 ORSA IV (CA-MRSA** in the US), Archaic/Iberian, ST250 ORSA I
t002	5.99 %	Austria, Belgium, China, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Iceland, Israel, Italy, Japan, Lebanon, Netherlands, Norway, Romania, Sweden, Switzerland, Taiwan, United Kingdom, United States	CC001	ST-5, ST-231	CC5, Rhine Hesse MRSA (prototype), EMRSA-3*, New York clone, Japan clone, Pediatric, USA100 ORSA II, USA800 ORSA IV, ST 5 ORSA I
t037	3.32 %	Austria, Bulgaria, China, Croatia, Czech Republic, Denmark, France, Germany, Italy, Lebanon, Netherlands, Norway, Poland, South Africa, Sweden, Switzerland, Taiwan, United Kingdom	CC037	ST-239, ST-240, ST-241	CC8/239, Vienna MRSA, Brazilian/Hungarian, ST239 ORSA III, ST240 ORSA III, EMRSA-1*, -4, -7, -9, -11
t044	2.50 %	Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Hungary, Italy, Lebanon, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom	CC044	ST-80	CA-MRSA** (<i>lukS</i> - <i>lukF</i> +) (CA-MRSA in Europe)
t011	2.19 %	Austria, Belgium, France, Germany, Netherlands, Norway	CC037	ST-398	Pig-associated clone
t001	2.12 %	Austria, Belgium, Croatia, Germany, Israel, Italy, Netherlands, Norway, Slovenia, South Africa, Sweden, Switzerland, United Kingdom, United States	CC001	ST-5, ST-222, ST-228	CC5, Southern German MRSA (prototype & subclone), Rhine Hesse MRSA (subclone), EMRSA-3*, New York clone
t004	1.62 %	Belgium, Denmark, Finland, France, Germany, Israel, Netherlands, Norway, Sweden, Switzerland, United States	CC004	ST-45	CC45, Berlin MRSA (prototype), USA600 ORSA II, USA600 ORSA IV
t015	1.45 %	Austria, Belgium, Croatia, Denmark, Finland, Germany, Indonesia, Italy, Latvia, Netherlands, Norway, Romania, South Africa, Spain, Sweden, Switzerland, Taiwan, United Kingdom	CC015	ST-45	

* EMRSA = Epidemic methicillin-resistant *Staphylococcus aureus*

** CA-MRSA = Community-Acquired methicillin-resistant *Staphylococcus aureus*

first line typing method, besides other typing methods, such as MLST and PFGE. In the following, the most important decisions of SeqNet.org plenary meeting are described.

External quality control standard and proficiency testing

Annual proficiency tests for all SeqNet.org participating laboratories will be performed. Currently only submission of single primer-based results are possible if excellent quality is ensured. Less than five edits are allowed, otherwise no synchronisation with the server is possible. With the existing quality criteria, 99.7% of all current submissions have the maximum quality value of 120 out of 120 (excellent).

Spa server as a common strain pool

The *spa* server provides information on strains originating from various human and veterinary specimens isolated in countries all over Europe and other parts of the world. As it might therefore serve as a decentralised worldwide virtual strain collection, the server is programmed to be searchable for basic information (*spa* type, MRSA/MSSA, PVL, infection/colonisation, human/animal origin). An anonymous strain request system will be available in 2008.

Unique nomenclature

SeqNet.org maintains the curatorship of the *spa* server and is the gatekeeper for all bioinformatic tools using the *spa* server for synchronisation of data. Users of other *spa*-analysing software tools have the possibility to synchronise with the *spa* server, provided they fulfil all given quality criteria. Up to now, agreements have been achieved between SeqNet.org and two developers of *spa*-analysing softwares (Ridom: <http://www.ridom.de> and Applied Maths: <http://www.applied-maths.com>). All users of the *spa* server are invited to perform the SeqNet.org certification and annual proficiency test.

SeqNet.org membership, management, organisation and data flow and data property

As local and regional laboratories submit data to the *spa* server, this might lead to a bypassing of national reference laboratories (NRL) which are responsible for regional and especially national molecular surveillance and public health action. A technical software solution could make it possible that, upon mutual agreement between NRL and the local laboratories, all epidemiologically interesting *spa*-typing data could be used in future by the NRL. National or regional data can be made visible by the national reference laboratory. Even cross-border, euregional data can be made visible as it has been done for regional networks such as EUREGIO MRSA-net Twente, Münsterland (<http://www.mrsa-net.eu>). Nevertheless, it remains up to national initiative to build up such national *spa*-typing networks. It is important to mention that all data on the *spa* server is strictly incrementally synchronised. This means that all synchronised data after having passed quality control and assignment of the *spa* type is stored with a single laboratory identifier. Every submitter using direct submission is able to withdraw his/her data at any time by re-synchronising with the server and indicating the deletion of the submission. Only the *spa* type and the information on quality will remain on the server. International study groups or regional and national networks can choose the option of not making visible their data submission (again, except *spa* type and quality) on the public homepage as long as they wish. In this way, data property of each single submitter is assured at any time.

Collaboration

There is collaboration with other European networks, such as the European Antimicrobial Resistance Surveillance System ([earss.nl\). Most of the national reference laboratories are involved in both networks \[15\]. Furthermore, many members of the HARMONY IUMS are also members of SeqNet.org \[16,17\]. In 2007, SeqNet.org started a collaboration with the Nosocomial Infection Control Consortium \(INICC, <http://www.inicc.org>\) in Argentina, a worldwide network for surveillance of nosocomial infections in developing countries. Most of these countries are interested in external quality control ring-trials for their national laboratories and seek assistance for typing methods. The collaboration with SeqNet.org can foster collaboration between epidemiologists and microbiologists in those countries.](http://www.</p></div><div data-bbox=)

Conclusion

The SeqNet.org initiative is a vivid European-wide network of laboratories for sequence-based typing of microbial pathogens, especially *S. aureus*. It generates high-quality typing data available to all participants and the public through the web-portal. The SeqNet.org laboratory network delivers the tools to detect local, national and international spread of MSSA and MRSA *spa* types. In particular, real-time synchronisation, automatic quality control and data property have made the SeqNet.org curatorship of the *spa* server successful for many years. In consequence, there is a strong need for EU-wide standardised sampling regimen to improve the use of *spa* type data for epidemiological purposes, such as the interpretation of relative frequencies, the time frame, and the geographical spread of *S. aureus spa* types. As the method is employed by both human and veterinary laboratories, typing results can already be used today for interdisciplinary epidemiological studies of MRSA. More laboratories from all parts of the world are welcome to join this initiative. If you are interested in joining SeqNet.org, please contact the coordinators.

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