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Exotoxin-encoding gene content in community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus*

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Abstract

Reports of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) causing hospital infections are increasing, and it is questionable whether the existing molecular definition of CA-MRSA is suitable for the characterization of all strains involved. The 821 methicillin-resistant *S. aureus* (MRSA) isolates recovered from patients in Health Region East, Norway during the period 1991–2006 were characterized by multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylococcal protein A (*spa*) gene typing, and their content of exotoxin-encoding genes. Cluster analysis based on exotoxin-encoding gene content was performed to separate the MRSA isolates into valid clusters with respect to microbiological characteristics. The analysis gave a four-cluster structure, and the four toxin clusters differed in the genetic lineages they included and in the diversity of the genetic lineages. A few genetic lineages were present in several toxin clusters. These results support the theory that mobile genetic elements encoding virulence genes do not move randomly among genetic lineages, but are restricted by the clonal lineages' genetic background. Using the molecular criteria, MLST type, SCC*mec* type and the presence of the *lucS/F*-Panton–Valentine leukocidin (PVL) gene to define a CA-MRSA isolate, it was found that the CA-MRSA isolates mainly grouped together in two toxin clusters with a low prevalence of exotoxin-encoding genes. Statistical analyses supported the conclusion that toxin clusters with CA-MRSA genetic lineages were characterized by a low prevalence of exotoxin-encoding genes, whereas toxin clusters with hospital-acquired MRSA genetic lineages were characterized by a higher prevalence of exotoxin-encoding genes.

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Introduction

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains have been found to differ genetically from hospital-acquired MRSA (HA-MRSA) strains in three major genotypic markers: their genetic lineage, the staphylococcal cassette chromosome *mec* (SCC*mec*) type, and the presence of the *lucS/F*-Panton–Valentine leukocidin genes (PVL) [1]. The CA-MRSA strains are reported to have a more diverse genetic background than HA-MRSA strains [2,3]. Some strains are closely related to HA-MRSA, but most have genetic characteristics not found in HA-MRSA [4]. Distinguishing CA-MRSA from HA-MRSA on epidemiological grounds may be difficult, as detailed information on the patient's background and medical history has to be obtained.

Defining methicillin-resistant *S. aureus* (MRSA) as community-acquired on the basis of the isolate's genetic content has been proposed as an alternative to the use of epidemiological criteria, and the molecular markers used are the genetic lineage, the SCC*mec* type, and the presence of the *lucS/F*-PVL genes. The prevalence of MRSA meeting the molecular criteria for being community-acquired is increasing inside hospitals, and MRSA strains not meeting the criteria are appearing in community infections, raising the question of whether there is a need for other or additional molecular

markers. There are reasons to believe that the selection pressures acting on the clones in the hospital environment are different from those prevailing in the community. Virulence-associated genetic elements might be affected differently, and it has been suggested that CA-MRSA and HA-MRSA differ in other parts of their exotoxin-encoding gene content [5]. In this study, we addressed the question of whether the exotoxin-encoding gene content could separate MRSA isolates into relevant groups or clusters.

We have a complete record of people infected with MRSA in the largest health region in Norway during the period 1991–2006, and the MRSA isolates obtained from these patients. The MRSA isolates have been characterized by sequence type (ST), SCC*mec* type, staphylococcal protein A type, and the presence or absence of several exotoxin-encoding genes. The epidemiological data available are limited to the information that accompanied the specimens to the laboratories.

Materials and Methods

Study population and bacterial isolates

This study was population-based and bi-directional, retrospective from 1991 to 2004 and prospective from 2005 to 2006, and included all people diagnosed with MRSA in Health Region East, a region covering 35% of the Norwegian population.

One MRSA isolate per person was included, except when several genetically distinct isolates had been obtained, in which case one isolate from each genotype was selected. The isolates were obtained from six microbiology laboratories.

MRSA identification and DNA isolation

All MRSA isolates were identified by growth on Mueller–Hinton agar containing 2% NaCl, 4 mg/L oxacillin and 8 mg/L aztreonam for 2 days, or by growth on chromID MRSA according to the manufacturer's instructions (bioMérieux SA, Marcy l'Etoile, France). The isolates were tested for oxacillin susceptibility by E-test (AB Biodisk, Solna, Sweden), and for the presence of the genes *mecA* and *SA442* using light cycler PCR and Roche light cycler system-PCR (Roche Applied Science, Indianapolis, IN, USA) [6]. Total DNA was isolated as described previously [7].

Multilocus sequence typing and BURST analysis

Multilocus sequence typing was performed by a modification of the protocol of Enright *et al.* [7,8].

The relationship between the STs was analysed using the clustering algorithm eBURST v/3 [9] (http://eburst.mlst.net), employing the group definition of similarity at six of seven loci; that is, members of a group might differ at a single locus.

SCC*mec* typing

SCC*mec* typing was performed by identifying the *ccr* and *mec* complexes. For SCC*mec* types I–IV, the typing was performed as described previously [7]. For SCC*mec* type V, the primers used for identification of the class C2 *mec* complex were IS-2 and mA2 [3], and those used for the *ccrC* complex were γF and γR [10]. These primers and the PCR conditions are described in Appendix S1.

Staphylococcal protein A (*spa*) typing and BURP analysis

Spa typing was performed by sequencing of the variable X region at the 3′-end of the *spa* gene, as described previously [7]. *Spa* types and BURP *spa* clonal complexes (*spa*-CCs) [11] were assigned using Ridom StaphType software v/1.5.5, situated on the SpaServer Typing website (http://spaserver.ridom.de/) [12]. The calculated cost among members of a group in this study was set to be less than or equal to four. *Spa* types shorter than five repeats were excluded from the analysis because no reliable deduction about ancestries could be made.

Exotoxin-encoding genes

The presence of genes coding for PVL (*lucS/F*-PVL), eight staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *sei*, and *sej*), three exfoliative toxins (*eta*, *etb*, and *etd*), an epidermal cell differentiation inhibitor (*edin-B*), toxic shock syndrome-1 (*tst*) and α-haemolysin (*hla*) was determined. The detection of staphylococcal enterotoxin genes, *eta*, *etb*, *tst* and *lucS/F*-PVL [13] was performed using real-time PCR and power SYBR Green master mix, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The detection of *hla* [14] and *etd* and *edin-B* [15] was performed by conventional PCR. The primers and the PCR conditions are described in Appendix S1.

Reference strains

The reference strains used were FRI913 (*sea*, *sec*, *see*, and *hla*), FRI1151m (*sed* and *sej*), RN4850 (*eta* and *etb*), 31844 (*etd* and *edin-B*) (in-house strain), CCUG2354 (*tst*), CCUG25924 (*seb*), CCUG47167 (*seg*, *sei*, and *lukS/F*-PVL), COL (SCC*mec*I), BK2464 (SCC*mec*II), ANS46 (SCC*mec*III), 44110 (SCC*mec*IV) (in-house strain), and WIS (SCC*mec*V).

Strains FRI913, FRI1151m, RN4850, COL, BK2464 and ANS46 were kindly provided by J. Etienne (Lyon, France). Strain WIS was kindly provided by T. Ito (Juntendo, Japan).

Splitting of database

To avoid the undesirable situation where hypotheses are derived after data have been seen, we used a data-splitting approach [16]. The MRSA database was randomly split into

two approximately equal parts after stratifying the entire dataset by the following three variables: age when infected, type of infection, and body site from which the bacterial sample was taken. One part of the data was used for identifying hypotheses, while the investigators remained blind to the second part. The second part was used for hypothesis testing. For the supported hypotheses, the entire dataset was used to obtain the final estimates.

All statistical analyses were performed following the data-splitting approach.

Statistical analyses

Statistical analyses were performed using SPSS v/15 for Windows and Microsoft Office Excel for Windows 2000.

To find possible microbiological characteristics that would divide isolates into relevant categories, cluster analysis based on the presence of the exotoxin-encoding genes *lukS/F*-PVL, *sea-see*, *seg*, *sei-sej*, *eta*, *etb*, *etd*, *tst* and *hla* in the MRSA isolates was performed. *Edin-B* was not used, as it occurred in association with *etd*. We applied hierarchical cluster analysis [17], where a dissimilarity matrix was computed using squared Euclidean distance, and clustering was performed by Ward's method [18]. Comparison of identified toxin clusters was performed using a chi-square test. A significance level of 5% was chosen.

Research ethics

The collection and analysis of data were approved by the Norwegian Social Science Data Services. The Regional Committee for Medical Research Ethics, Eastern Norway, approved the execution of the project as it was described in the application.

Results

Eight hundred and twenty-one people were infected or colonized with MRSA in the time period studied: 454 infections/colonizations were detected in healthcare institutions, 363 were detected outside such institutions, and for four people this information could not be retrieved.

MRSA genetic lineages

Several genetic lineages were found in the MRSA database (Table 1). Thirty-one *spa* types were not placed in a *spa*-CC by the BURP analysis. Seventeen were excluded because of short sequences, 12 were singletons, and two types were found to be related, although no founder could be predicted. For one MRSA isolate, the *spa* type could not be identified. Nine MRSA isolates were not of SCC*mec* type I–V: three were non-typeable, and in six isolates only one of the complexes was found.

TABLE 1. Methicillin-resistant *Staphylococcus aureus* (MRSA) genetic lineages (defined by clonal complex (CC), sequence type (ST), staphylococcal cassette chromosome *mec* (SCC*mec*) type, and *spa*-CC) encountered in the MRSA database of Akershus University Hospital Trust, covering Health Region East, Norway, during the period 1991–2006

CC[a] (*n*)	*Spa*-CC[b] (*n*)	ST-SCC*mec*[c]
1 (11)	127 (9)	1-IV, 474-V, 891-IV, 993-IV
	S (2)	1-IV, 772-V
5 (114)	002 (107)	5-I, 5-II, 5-IV, 5-V, 105-II, 114-II, 125-IV, 146-IV, 149-IV, 225-II, 231-II, 823-II, 826-IV, 998-IV
	062 (5)	5-I, 5-II, 5-IV
	E (2)	5-IV
8 (324)	008 (245)	8-IV, 8-V, 8-*mecA*, 8-*C2mec*, 94-IV, 112-I, 113-IV, 247-I, 254-I, 449-IV, 824-I, 995-IV, 999-IV
	012 (70)	110-*mecA*, 239-III, 241-III, 368-III, 592-III, 996-III
	148 (8)	72-IV, 72-V
	S (1)	72-IV
9 (1)	S (1)	834-IV
12 (5)	160 (5)	12-IV
15 (1)	S (1)	15-*ccrC*
22 (39)	022/032 (38)	22-IV
	E (1)	22-IV
30 (77)	012 (70)	30-IV, 30-V, 36-II, 37-IV
	S (4)	30-IV, 39-II
	E (3)	30-IV
45 (72)	065 (9)	45-IV
	216 (1)	45-IV
	015/116 (35)	45-IV, 820-IV
	722/102 (18)	45-IV, 108-IV, 882-IV
	E (9)	45-IV, 45-*ccrC*, 45-NT, 46-IV
59 (20)	216 (20)	59-IV, 59-V, 338-V, 375-IV
80 (108)	639 (106)	80-IV, 822-IV, 994-IV, 997-IV
	E (1)	80-IV
	NT (1)	80-IV
88 (22)	186 (22)	88-IV, 825-IV, 859-IV
97 (1)	S (1)	97-IV
228 (6)	002 (6)	111-I, 228-I
398 (1)	S (1)	398-V
509 (1)	S (1)	207-V
913 (1)	E (1)	913-V
d140 (2)	NF (2)	140-IV
d152 (14)	355 (14)	152-V
d154 (1)	639 (1)	154-IV

[a]For ST140, ST152, and ST154, no predicted founder could be established using eBURST v/3.
[b]S, singleton (no related *spa* type could be found using BURP); E, excluded (the *spa* sequence contained fewer than five repeats and was excluded from the BURP analyses); NF, related *spa* types, but no predicted founder could be found using BURP; NT, non-typeable.
[c]*C2mec*, only the *C2 mec* complex could be found; *mecA*, only the *mec*-complex A could be found; *ccrC*, only the *ccr* complex C could be found; NT, non-typeable.

Cluster analysis

Frequency analysis on the exotoxin-encoding gene content resulted in exclusion of two genes, *see* (not detected) and *etb* (present in only one isolate), from the cluster analysis, which was performed on the remaining 12 genes, and resulted in a four-cluster structure. The distributions of the exotoxin-encoding genes in the four clusters were found to be significantly different ($p < 0.001$) (Table 2).

TABLE 2. The distributions of the exotoxin-encoding genes in the cluster groups

Exotoxin-encoding genes	Toxin cluster group I		Toxin cluster group II		Toxin cluster group III		Toxin cluster group IV	
	Present (%)	Absent (%)	Present (%)	Absent (%)	Present (%)	Absent (%)	Present (%)	Absent (%)
lucS/F-PVL	36.9	63.1	1.8	98.2	14.9	85.1	100	0
sea	30	70	97.3	2.7	7.3	92.7	0	100
seb	20.2	79.8	0	100	0.9	99.1	0	100
sec	2.4	97.6	0	100	23.1	76.9	0	100
sed	0	100	100	0	13.9	86.1	0	100
seg	0	100	0	100	100	0	0	100
sei	0	100	0	100	99.4	0.6	0	100
sej	0	100	100	0	16.1	83.9	0	100
eta	4.2	95.8	0	100	0	100	0	100
etd	0.3	99.7	0	100	0	100	100	0
hla	94.1	5.9	100	0	99.7	0.3	98.1	1.9
tst	0.3	99.7	0	100	16.1	83.9	0	100

Toxin clusters and genetic lineages

The four clusters were found to be different with regard to both the presence of genetic lineages (clonal complex (CC), ST, SCC*mec* type, *spa*-CC) and the diversity of the lineages (Table 3). Cluster I was the most heterogeneous cluster and cluster III the second most heterogeneous; clusters II and IV consisted of isolates belonging solely to one CC (Table 3).

Exotoxin-encoding genes and genetic lineages

Exotoxin-encoding genes are known to have different distributions within and among genetic lineages. The exotoxin-encoding gene distribution within the CCs in this study is shown in Table 4. Seven isolates belonging to CC45, ST45-IV and *spa*-CC722/102 contained *sej* but not *sed*, despite the two genes being located on the same plasmid. One reason for this result might be that the primers used for detection of *sed* were not able to attach to the template in this clone.

LukS/F-PVL, known as a marker for CA-MRSA, was found in all toxin clusters (Table 2).

Discussion

The cluster analysis produced four toxin groups (clusters I–IV) that differed significantly in their exotoxin-encoding gene distribution.

Toxin clusters II and IV were homogeneous, both in their genetic lineage and in their exotoxin-encoding gene content. Among the 17 CCs discovered in the database, only five were found in more than one cluster. Four of these five were found in two clusters, and one, CC8, was found in three clusters. The CCs appearing in two clusters had a different distribution of isolates between the clusters: one cluster contained one isolate, and the second cluster contained the remaining isolates. These results indicate that the genetic lineages, with the exception of CC8, correlate well with the toxin clusters, and support the theory that mobile genetic elements (MGEs) encoding virulence genes do not move randomly among genetic lineages, but are restricted by the clonal lineages' genetic backgrounds [19,20]. CC8 was found in three clusters and had a different distribution among the clusters. Cluster III contained a few isolates, whereas clusters I and II contained 204 and 110 isolates, respectively. Only two of the 17 STs, ST8 and ST239, appeared in several clusters. ST8 appeared in clusters I and II, and ST239 in clusters I and III. The distribution among several clusters indicates that this genetic lineage is diverse in its exotoxin-encoding gene content, as reported by Holtfreter *et al.* [20]. One reason for this diversity might be that CC8 is a large and diverse CC, consisting of several successful clones. In addition to the founder clone, ST8, several STs have diversified to produce their own single-locus variants. One of these successful clones is ST239, a clone with a mosaic chromosome consisting of ST8 and ST30 [21].

In cluster IV, and only in this cluster, we found the European CA-MRSA lineage, CC80 [22,23]. The presence of exotoxin-encoding genes in this cluster was uniform. *LukS/F*-PVL and *etd* were present in all isolates, *hla* was present in 98.1% of the isolates, and the remaining exotoxin-encoding genes were absent. This lack of exotoxin-encoding genes is concordant with earlier studies on ST80 [24]. The homogeneity in the exotoxin-encoding gene content seems to be a unique feature of the CC80 lineage, and could be a result of this genetic lineage being less prone to accepting MGEs encoding virulence genes through horizontal gene transfer. The ability to restrict the entrance of foreign MGEs would separate the lineage, with regard to exotoxin-encoding genes, from other MRSA genetic lineages, and thus might be a reason why the cluster analysis separated the CC in one cluster.

Cluster II consisted of isolates within CC8, ST8 and *spa*-CC008. In the health region, there have been several outbreaks at long-term-care facilities with isolates belonging to

TABLE 3. **Methicillin-resistant *Staphylococcus aureus* genetic lineages (defined by clonal complex (CC), sequence type (ST), staphylococcal cassette chromosome *mec* (SCC*mec*) type, and *spa*-CC) in the four toxin cluster groups**

Cluster group (*n*)	CC (*n*)	*Spa*-CC[b] (*n*)	ST-SCC*mec*[c]
I (287)	1 (10)	127 (9)	1-IV, 474-V, 891-IV, 993-IV
		S (1)	1-IV
	5 (1)	062 (1)	5-IV
	8 (204)	008 (135)	8-IV, 8-*mecA*, 8-V, 94-IV, 112-I, 113-IV, 247-I, 254-I, 449-IV, 824-I, 995-IV, 999-IV
		012 (69)	110-*mecA*, 239-III, 241-III, 368-III, 592-III, 996-III
	9 (1)	S (1)	834-IV
	12 (5)	012 (5)	12-IV
	15 (1)	S (1)	15-*ccrC*
	45 (1)	216 (1)	45-IV
	59 (20)	216 (20)	59-IV, 59-V, 338-V, 375-IV
	88 (22)	186 (22)	88-IV, 825-IV, 859-IV
	97 (1)	S (1)	97-IV
	228 (1)	002 (1)	228-I
	398 (1)	S (1)	398-V
	509 (1)	S (1)	207-V
	913 (1)	E (1)	913-V
	d140[a] (2)	NF (2)	140-IV
	d152[a] (14)	355 (14)	152-V
	d154[a] (1)	639 (1)	154-IV
II (110)	8 (110)	008 (110)	8-IV, 8-V, 8-*C2mec*
III (316)	1 (1)	S (1)	772-V
	5 (113)	002 (103)	5-I, 5-II, 5-IV, 5-V, 105-II, 114-II, 125-IV, 146-IV, 149-IV, 225-II, 231-II, 823-II, 826-IV, 998-IV
		062 (4)	5-I, 5-II, 5-IV
		E (2)	5-IV
	8 (10)	012 (1)	239-III
		148 (8)	72-IV, 72-V
		S (1)	72-IV
	22 (39)	022/032 (38)	22-IV
		E (1)	22-IV
	30 (77)	012 (70)	30-IV, 36-II, 37-IV
		S (4)	30-IV, 39-II
		E (3)	30-IV
	45 (71)	065 (9)	45-IV
		015/116 (35)	45-IV, 45-V, 820-IV
		722/102 (18)	45-IV, 108-IV, 882-IV
		E (9)	45-IV, 45-*ccrC*, 45-NT, 46-IV
	228 (5)	002 (5)	111-I, 228-I
IV (108)	80 (108)	639 (106)	80-IV, 822-IV, 994-IV, 997-IV
		NT (1)	80-IV
		E (1)	80-IV

[a]No predicted founder could be found using eBURST v/3.
[b]S, singleton (no related *spa* type could be found using BURP); E, excluded (the *spa* sequence contained fewer than five repeats and was excluded from the BURP analyses); NF, related *spa* types but no predicted founder could be established using BURP; NT, non-typeable.
[c]*C2mec*, only the *C2 mec* complex could be found; *mecA*, only the *mec*-complex A could be found; *ccrC*, only the *ccr* complex C could be found; NT, non-typeable.

TABLE 4. **Distribution of exotoxin-encoding genes within the methicillin-resistant *Staphylococcus aureus* (MRSA) genetic lineages (defined by clonal complex (CC), or sequence type (ST) if no CC could be found) discovered in Health Region East, Norway**

Exotoxin-encoding gene	Genetic lineage
sea	CC1, CC5, CC8, CC30, CC59, CC228
seb	CC5, CC8, CC12, CC59
sec	CC1, CC5, CC8, CC9, CC22, CC30, CC45, ST154
sed	CC5, CC8
seg	CC1, CC5, CC8, CC22, CC30, CC45, CC228,
sei	CC1, CC5, CC8, CC22, CC30, CC45, CC228,
sej	CC5, CC8, CC45
eta	CC88, CC913
etd	CC80, CC913
tst	CC5, CC8, CC22, CC30, CC45
hla	CC1, CC5, CC8, CC9, CC12, CC15, CC22, CC30, CC45, CC59, CC80, CC88, CC97, CC228, CC398, CC509, CC913, ST140, ST152, ST154
lukS/F-PVL	CC1, CC5, CC8, CC9, CC30, CC59, CC80, CC398, ST152, ST154

ST8-IV and *spa*-CC008. It is therefore possible that the clone was over-represented in our MRSA database, which could influence the cluster analysis. The exotoxin-encoding gene content in cluster II was almost as homogeneous as that in cluster IV, with only *lukS/F*-PVL and *sea* not having 100% presence or absence. The exotoxin-encoding genes present were *lukS/F*-PVL, *sea*, *sed*, *sej*, and *hla*.

In cluster I, CC8 was the largest CC, and the majority of the isolates in CC8 were identical, on the ST, SCC*mec* and *spa*-CC level, to the isolates in cluster II, but the cluster analysis split these isolates into two clusters, where the *lukS/F*-PVL-positive isolates were gathered in one cluster and the negative isolates in a second cluster. In cluster I, the majority of the ST8-IV and *spa*-CC008 isolates contained *lukS/F*-PVL and *hla*, compatible with the ST8:USA300 clone. In cluster II, the majority of the isolates did not harbour *lukS/F*-PVL, but did harbour *sed*, *sej*, and *hla*. Diep *et al.* [25] found that strains belonging to the ST8 lineage possessed similar virulence gene repertoires. The discrepancy between our results and those of Diep *et al.* might have resulted from our examining fewer determinants. Among the other genetic lineages found in cluster I, both HA-MRSA and CA-MRSA clonal lineages were represented (Table 3).

On the basis of genetic lineages and the number of *lukS/F*-PVL-positive isolates, we propose that a majority of CA-MRSA genetic lineages were grouped in cluster I according to a similar exotoxin-encoding gene repertoire, and that this repertoire is shared with some HA-MRSA lineages. The presence of exotoxin-encoding genes in cluster I was relatively low. Eight of the 12 exotoxin-encoding genes were represented in less than 5% of the isolates, with four being absent. The CA-MRSA clonal lineages thus seem to be found in clusters with a low prevalence of exotoxin-encoding genes, with only one exception, the Oceania clone ST30-IV, present in cluster III.

Cluster III was represented by four large and three small CCs, and major pandemic and epidemic HA-MRSA clones were present (Table 3). This cluster contained the highest percentage of exotoxin-encoding genes, but *lukS/F*-PVL was primarily found in the Oceania clone. Most of the pandemic and epidemic HA-MRSA clones were clustered together on

the basis of their exotoxin-encoding gene content, and they clustered with one large CA-MRSA clone.

All MRSA risk factors, as described by the US CDC (http://www.cdc.gov), could not be identified in this study, and this could have resulted in an overestimation of CA-MRSA. This would not alter our main conclusions. The study was bi-directional, retrospective from 1991 to 2004 and prospective from 2005 to 2006. However, the criteria used in primary care or in healthcare institutions for taking MRSA samples were not influenced by the investigation taking place. All MRSA isolates identified by the microbiological laboratories were included, and we believe that the change in case ascertainment has had a minimal influence on our results and conclusions.

To conclude, we have demonstrated four MRSA toxin clusters, based on a cluster analysis of the exotoxin-encoding genes of 821 MRSA isolates. A few genetic lineages were split into several clusters, supporting the theory of a certain restriction in the movement of MGEs encoding virulence genes among different genetic lineages. CA-MRSA clones tended to group together in clusters defined by a low prevalence of exotoxin-encoding genes, and HA-MRSA clones tended to group together in clusters with a higher prevalence of exotoxin-encoding genes.

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Transparency Declaration

This work was supported by grants from Eastern Norway Regional Health Authority, Norway. The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Oligonucleotide primers and reference strains used for detection of exotoxin-encoding genes and SCC*mec* type V.

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