Epidemiology of European Community-Associated Methicillin-Resistant \textit{Staphylococcus aureus} Clonal Complex 80 Type IV Strains Isolated in Denmark from 1993 to 2004\(^\text{\dag}\)

A. R. Larsen,\(^1\) S. Böcher,\(^1\) M. Stegger,\(^1\) R. Goering,\(^2\) L. V. Pallesen,\(^1\) and R. Skov\(^1\)

National Center for Antimicrobials and Infection Control, Statens Serum Institut, Copenhagen, Denmark,\(^1\) and Department of Medical Microbiology and Immunology, Saint Joseph Hospital, Creighton University Medical Center, Omaha, Nebraska\(^2\)

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In Europe, community-associated methicillin-resistant \textit{Staphylococcus aureus} (CA-MRSA) infections have been caused predominantly by isolates belonging to the “European CA-MRSA” clone (sequence type 80, staphylococcal cassette chromosome \textit{mec} type IV). In this study, the epidemiology of European CA-MRSA was investigated on a nationwide scale, covering the period from 1993 to 2004. Denmark has been a low-prevalence country regarding MRSA since the mid-1970s but has experienced an increase in the number of new MRSA cases in recent years. Our results show that European CA-MRSA contributed to this increase. The isolates primarily caused skin and soft tissue infections (SSTIs) in patients outside hospitals, and transmission between household members was the predominant mode of spread. Although some of the isolates were found in hospitalized patients, nosocomial transmission seemed likely in only one instance, pointing to endogenous infections as an important factor. Compared to the CA-MRSA clone most common in the United States (USA300), the European CA-MRSA clone seems less well adapted to persist in hospital environments. Patients with a recent history of travel or family relation to the Mediterranean or Middle East were highly overrepresented. The epidemiological data indicated that the European CA-MRSA isolates were introduced into Denmark on multiple occasions, paralleled by an increasing level of genetic diversity of the isolates found during the study period. European CA-MRSA has previously been described as a rather uniform clone. However, we found pronounced, diverse pulsed-field gel electrophoresis subtypes, staphylococcal protein A gene (spa) types, and susceptibility patterns.

The emergence of community-associated methicillin-resistant \textit{Staphylococcus aureus} (CA-MRSA) has caused a change in MRSA epidemiology and an increasing number of MRSA cases in many areas, including previously low-incidence countries, such as the Nordic countries and The Netherlands. CA-MRSA isolates primarily cause skin and soft tissue infections (SSTIs), but invasive infections such as bacteremia and necrotizing pneumonia have also been reported (10, 19). CA-MRSA clones have traditionally been characterized based on pulsed-field gel electrophoresis (PFGE) typing and named according to their geographic distribution, e.g., South Pacific CA-MRSA, USA300 and USA400 CA-MRSA clones, etc.

In Europe, most CA-MRSA isolates have been associated with multilocus sequence type (MLST) clonal complex 80 sequence type 80 (CC80:ST80) and with staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}) type IV (1, 5, 12, 36, 47) and are referred to hereafter as CC80-MRSA-IV isolates (41). CC80-MRSA-IV isolates differ from other CA-MRSA strains by often being resistant to fusidic acid, with additional characteristic resistance to tetracycline, streptomycin, and kanamycin (TSKF profile) but with susceptibility to gentamicin (8, 29, 47).

CC80-MRSA-IV isolates were first described in Denmark as EDK-97 (39a) but have since been found in most European countries, e.g., Greece, Finland, France, Germany, The Netherlands, Norway, and Sweden (1, 5, 12, 36, 47). In Denmark, \textit{Staphylococcus aureus} infections have been surveyed by the Staphylococcus Laboratory, Statens Serum Institut (SSI), since 1957. Since 1988, all MRSA isolates have been referred prospectively to the Staphylococcus Laboratory, where they have been typed, susceptibility tested, and stored. Furthermore, patient data have been retrieved systematically from general practitioners and hospital discharge summaries since 1999. Denmark has been a low-prevalence country since the mid-1970s, with a prevalence of MRSA of <1% for bacteremia isolates (annual report on \textit{Staphylococcus aureus} bacteraemia in Denmark [http://www.ssi.dk/sw621.asp]). However, since the late 1990s, an increase in new MRSA cases has been observed, especially involving community-associated infections.

CC80-MRSA-IV isolates have been tracked retrospectively back to 1993 in the national MRSA collection at SSI (8, 42). Thus, the objective of this study was to describe the epidemiology of the European CA-MRSA clone in Denmark during the period from 1993 to 2004.

**MATERIALS AND METHODS**

\textbf{Isolates.} The first MRSA isolate from each patient or healthy carrier found in Denmark from 1999 to 2004 was subjected to PFGE typing (\(n = 1,143\)). Isolates with \(\geqslant80\%\) similarity in PFGE profile (see below) to typical isolates of CC80-MRSA-IV were included in this study, yielding a total of 255 isolates (26).

Furthermore, the MRSA database was screened for possible CC80 isolates prior to 1998 (1988–1998), based on the characteristic and unusual antibiogram (TSKF profile) with susceptibility to gentamicin. Isolates with this resistance...
pattern were typed by PFGE followed by SCCmec typing. This resulted in inclusion of an additional 39 CC80-MRSA-IV isolates in the study.

**mecA** confirmation and antibiotic susceptibility testing. The presence of mecA and mcr genes in all MRSA isolates was confirmed using an Evigen MRSA detection kit following the manufacturer’s instructions (SSI, Copenhagen, Denmark) (39). Susceptibility tests for penicillin, cefoxitin, streptomycin, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline, fusidic acid, rifampin, and ciprofloxacin were performed using Neosensitabs (Rosco, Taastrup, Denmark) on Danish blood agar (SSI, Copenhagen, Denmark), using a semiconfluent (10³ CFU/ml) inoculum and overnight incubation in ambient air at 35 to 36°C. Susceptibility to fusidic acid was confirmed by determining twofold agar dilution MICs on Mueller-Hinton agar, with an inoculum of 10⁴ CFU/ml and incubation at 35 to 36°C. Resistance was defined as an MIC of >1 mg/liter (38, 41).

PCR confirmation of the presence of the fusidic acid resistance gene, fuscB, was determined as previously described (35).

**Clinical and epidemiological information.** Clinical and epidemiological information for all patients was obtained retrospectively from discharge summaries and notes from general practitioners registering the following clinical and epidemiological data: reason for specimen collection (infection or screening), infected body site (skin and soft tissue, blood, respiratory tract, bone/joint, urinary tract, postoperative wound, etc.), hospitalization or residence in a long-term care facility in the 12 months prior to diagnosis, household members with a history of MRSA colonization or infection, ethnicity, family relation to foreign countries, history of travel in direct relation to diagnosis of MRSA, and other factors deemed of significance by the reporting physician. Based on the patient data, infections were categorized into the following four different groups, as described by Klevens et al. (20): imported, health care associated (HA), health care associated with a community onset (HACO), and CA. The following definitions were used: (i) imported infections were infections acquired outside Denmark; (ii) HA infections had MRSA isolated in hospitals more than 48 h after admission, without signs of staphylococcal infection at admission; (iii) HACO infections had MRSA isolated outside hospitals or less than 48 h after admission from patients who were either hospitalized within the last 12 months to infection, residents at a nursing home, or health care workers; and (iv) CA infections had MRSA isolated at general practitioner offices or less than 48 h after admission to a hospital from patients without direct contact to hospitals or nursing homes for 12 months prior to infection.

**PFGE typing.** All isolates were subjected to PFGE analysis according to the Harmony protocol (31) and were analyzed by BioNumerics software, version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium), using Dice coefficients and the unweighted-pair group method using average linkages. A similarity coefficient of ≥80% was used to define the CC80-MRSA-IV cluster, which also included seven international European CA-MRSA isolates (26). In order to confirm the PFGE cluster as CC80, 55 isolates were typed by spa typing and 10 isolates were typed by MLST.

Based on the general uniformity of CC80-MRSA-IV PFGE patterns, isolate subtypes were defined as having one or more PFGE band difference, similar to the approach taken by others working with organisms exhibiting highly conserved PFGE profiles (3). On this basis, a total of 55 representative isolates from the various subtypes were selected and further characterized.

**spa typing and MLST.** Typing of the protein A gene (spa) and MLST were performed as previously described (14). The spa types and STs were assigned using Ridom StaphType (version 1.4.11) and the MLST database (http://www.mlst.net), respectively.

**Accessory global regulator (agr) typing.** agr types I to IV were determined as previously described by Jarraud et al. (18). S. aureus strains RN1, RN6607, RN3984, and RN4850 were included as controls for agr types I, II, III, and IV, respectively.

**SCCmec and dru typing.** SCCmec types I to IV were determined by the multiplex PCR strategy described by Oliveira and de Lencastre (34). Isolates not designated using this method as well as all subtype representatives were investigated by PCR detecting the recombinase gene ccrA, ccrB, or ccrC (17, 33) and by a multiplex PCR distinguishing SCCmec subtypes IVa to IVg (28).

The mec-associated direct repeat unit (dru), previously used to subdivide apparently clonal isolates of ST22 (EMRSA-15) in Scotland (11), was also studied. Amplification of dru sequences utilized the following primers: dru FW (5’ GGTTAGCATATTACCTCTTCCGTG) and dru Rev (5’ GCCGATTGTGCTTGATAG). Amplicons were obtained using AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) and the following PCR conditions: initial denaturation step at 94°C for 7 min followed by 30 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. The obtained sequences were compared to the 40-bp dru repeat consensus sequence (5’ ATAAAGGGTACGTAAAGCACG TTCTAAGTAAATTTGCAG) (32).

**Detection of toxin genes.** Detection of the Panton-Valentine leukocidin (PVL)-encoding genes ( lukS-PV and lukF-PV) and the exfoliative toxin D gene (etd) was performed by PCR as previously described (7, 49). A multiplex PCR was used for detection of the tst, eta, and etb genes, encoding staphylococcal toxic shock syndrome toxin 1, exfoliative toxin A, and exfoliative toxin B, respectively (27). Two multiplex PCRs were performed for detection of the staphylococcal enterotoxin genes sea-see and seg-nej (4).

**Statistics.** Statistical analysis was done by chi-square, Mann-Whitney, and one-way analysis of variance tests, with P values of <0.05 indicating significance.

## RESULTS

From 1988 to 2004, a total of 294 isolates with a PFGE pattern included in the CC80-MRSA-IV cluster was found. The first isolate was found in 1993, and until 1997, only sporadic cases (one to four per year) of CC80-MRSA-IV infection were encountered. Thereafter, the incidence of isolates increased from 8 in 1997 to 50 in 2001, with the latter representing 47.6% of all new MRSA cases in 2001. In the years 2002 to 2004, the number of new CC80-MRSA-IV cases was 26, 41, and 88, respectively (Fig. 1). However, due to the steep increase in the total number of MRSA isolates in Denmark over this time period, the relative proportion of CC80-MRSA-IV isolates declined to 16.1% in 2004. Throughout this time period, CC80-MRSA-IV isolates disseminated to an increasing number of counties, and by 2004, they were widespread throughout the country.

**Clinical characteristics.** The clinical data are presented in Table 1. The median age of the CA-MRSA patients (n = 133) was 26 years, which was significantly lower (one-way analysis of variance; P < 0.0001) than those for HA-MRSA (n = 18; median age, 55.5 years) and HACO-MRSA (n = 50; median age, 33 years) patients. Furthermore, the HA-MRSA infections caused by CC80-MRSA-IV affected younger patients than those infected with other types of MRSA in the same period (median age, 55.5 years versus 66 years [n = 234]; Mann-Whitney P = 0.025).

As shown in Table 1, 209 of the 242 infections (86.4%) were isolated from SSTIs, with abscesses being the most frequent clinical manifestation (82%), while cases of impetigo, furunculosis, carbunculosis, and atopic dermatitis constituted other clinical manifestations.

Invasive infections were encountered in eight patients (3.3%), including seven cases of bacteremia and one patient.
with pneumonia. In three instances, the bacteremia had a community onset.

**Onset and source of infections.** In 242 of 294 cases (82.3%), CC80-MRSA-IV was detected as an infection, while 46 cases (15.6%) were found by active screening (Table 1). In six cases, no clinical data were available. Among infections, 20 cases (8.2%) were acquired abroad. Of the 222 domestic infections, 18 cases (8.1%) were HA, 50 (22.5%) cases were diagnosed outside hospitals but had health care-related risk factors (HACO), and 133 (59.9%) cases were diagnosed in the community from patients without health care-related risk factors (CA). In the remaining 21 cases (9.5%), data on acquisition were missing (primarily for patients from the beginning of the study period).

Direct transmission between hospitalized patients seemed likely in only one case involving two patients situated at the same hospital for overlapping periods, with isolates sharing the same PFGE subtype (A1) and resistance profile. The remaining 66 cases of HA and HACO infections were scattered both in time and geographically, making the route of transmission unclear. Of the 133 CA cases, 41 patients had close contact with a known CC80-MRSA-IV carrier/patient, whereas in 92 CA cases no direct probable source of MRSA was identified. Of the 133 CA cases no direct probable source of MRSA was identified.

The epidemiological information revealed that 41% of CA and HACO infections affected individuals with a family history associated with foreign countries, including other regions/countries from those described above, e.g., the United States, Pakistan, and the Philippines.

Among the 20 imported cases, 5 were found in three different refugee camps, all of which had a relationship to the Balkans. The remaining 15 patients all had an association with the Mediterranean area (Egypt, Lebanon, Algiers, Malta, Greece, Cyprus, or Spain). During 1999 to 2004, MRSA infections were acquired abroad. Of the 222 domestic infections, 18 cases (8.1%) were HA, 50 (22.5%) cases were diagnosed outside hospitals but had health care-related risk factors (HACO), and 133 (59.9%) cases were diagnosed in the community from patients without health care-related risk factors (CA). In the remaining 21 cases (9.5%), data on acquisition were missing (primarily for patients from the beginning of the study period).

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### TABLE 1. Types of infections caused by CC80-MRSA-IV

| Infection site          | Imported (n = 20) | HA (n = 18) | HACO (n = 50) | CA (n = 133) | Unknown (n = 21) | Total (n = 242) |
|-------------------------|-----------------|------------|--------------|-------------|----------------|----------------|
| Skin and soft tissue    | 19              | 7          | 43           | 119         | 19             | 209            |
| Blood                   | 4               | 1          | 1            | 2           | 7              | 14             |
| Ear                     | 1               | 1          | 2            |             | 4              |                |
| Respiratory tract       | 1               | 1          | 2            |             | 4              |                |
| Deep seated             | 2               | 1          | 1            | 4           |                |                |
| Other                   | 1               | 4          | 3            | 4           | 2              | 14             |
| **Total**               | **21**          | **15**     | **56**       | **133**     | **21**         | **242**        |

The median (range) ages for patients with imported, HA, HACO, CA, unknown, and all infections were 19 (1 to 49), 37 (0 to 90), 33 (0 to 90), 26 (0 to 88), 20.5 (1 to 82), and 27 (0 to 90) years, respectively. n, number of patients.

### PFGE subtypes of CC80-MRSA-IV.** By PFGE, 17 different Smal band patterns (subtypes A1 to A17) could be distinguished during the study period (Fig. 2). Smal PFGE subtype A1 dominated throughout the period and was found in 218 cases (74.1%).

Between 1993 and 1995, only the A1 subtype was present, and thereafter, the number of different subtypes detected increased steadily each year during the study period, with 14 different subtypes observed in isolates from 2004.

Among the 20 imported cases, 14 (70%) had PFGE profile A1, with additional PFGE profiles A3, A4, A5, A7, A13, and A14 for single isolates.

Further typing and molecular characterization of 55 representatives of the 17 different PFGE patterns showed that all were agr type III and carried ets and pem but were negative for other toxin genes (eta, ets, and pem-sej). By spa typing, all isolates were found to be type t044 (47/55 isolates) or single-locus variants thereof (t131, t376, t435, t455, and t1109). spa types t131 and t376 were found in three cases each, and t1109 was found in two cases, whereas t435 and t455 were found on single occasions (Table 2). Different spa types did not correlate with specific PFGE subtypes.

**SCCmec typing.** SCCmec type IV was found in 256/294 (86.3%) isolates, whereas 38 isolates were nontypeable (NT) by the multiplex PCR described by Oliveira and de Lencastre (Table 2). Amplification of the recombinase genes (ccrA, ccrB, and ccrC) of the NT isolates and all subtype representatives failed for six of the isolates, whereas the remaining carried the ccrAB2 allele. Further PCR analysis by the method described by Milheirico et al. revealed that all isolates, including the NT isolates, carried SCCmec IVC (28). The observed variance obtained with the different SCCmec PCR strategies could not be linked to any epidemiological relationships between the isolates (Table 2).

Sequencing the mec-associated dru region of representatives from each of the 17 PFGE profiles revealed that they all carried dru type 10a.

**Susceptibility testing.** Complete antibiograms were obtained for 287/294 isolates (7 isolates were uncultivable/lost).

The predominant profile found for 172 of 287 isolates (60%) was the TSKF profile. Great diversity in susceptibility was observed for the remaining 115 isolates, yielding 28 different resistance profiles. The diversity was not correlated with specific PFGE subtypes, e.g., the TSKF profile was found in 11 of the subtypes and 20 different resistance profiles were encountered among isolates with the A1 PFGE subtype (Table 3).

All but 17 isolates (94.1%) were resistant to fusidic acid. The sensitivity to fusidic acid of these 17 susceptible isolates was confirmed by MIC testing. However, the fusB gene was present...
in 3 of the 17 isolates. There were no obvious epidemiological relationships between these three isolates. Eleven of the fucidic acid-susceptible isolates lacking the fusB gene were also susceptible to tetracycline. Resistance to fluoroquinolones was observed in 22 (7.7%) of the isolates, but the fluoroquinolone-resistant isolates were found predominantly in year 2001, especially in the county of Northern Jutland, but in cases without an obvious epidemiologic connection or correlation with onset of infections.

**DISCUSSION**

Although CA-MRSA isolates in Europe have been described extensively, only a few studies have described the epidemiology covering larger regions and longer time periods (9, 41, 46). Prospective collection of all MRSA strains isolated in Denmark combined with molecular typing and clinical information has enabled a detailed monitoring of the epidemiology of MRSA isolates on a nationwide scale. CC80-MRSA-IV

**TABLE 2. Characterization of CC80-MRSA-IV subtypes A1 to A17**

| CC80-MRSA-IV PFGE subtype | Total no. of isolates | No. of isolates tested | spa type(s) | SCCmec type | No. of different antibiograms$^d$ |
|---------------------------|-----------------------|------------------------|-------------|-------------|----------------------------------|
| A1                        | 218                   | 14                     | t044, t131, t376, t455 | IV, NT | IVC | 23 |
| A2                        | 6                     | 6                      | t044, t131 | IV, NT | IVC | 2 |
| A3                        | 5                     | 1                      | t044 | IV | IVC | 2 |
| A4                        | 8                     | 7                      | t044 | IV, NT | IVC | 2 |
| A5                        | 6                     | 1                      | t044 | IV | IVC | 2 |
| A6                        | 5                     | 1                      | t044 | IV | IVC | 2 |
| A7                        | 8                     | 3                      | t044 | IV | IVC | 2 |
| A8                        | 1                     | 1                      | t044 | IV | IVC | 2 |
| A9                        | 4                     | 3                      | t044, t1109 | IV | IVC | 2 |
| A10                       | 1                     | 1                      | t044 | IV | IVC | 2 |
| A11                       | 1                     | 1                      | t131 | IV | IVC | 2 |
| A12                       | 1                     | 1                      | t044 | IV | IVC | 2 |
| A13                       | 1                     | 1                      | t044, t376 | IV | IVC | 5 |
| A14                       | 1                     | 1                      | t044, t435 | IV, NT | IVC | 2 |
| A15                       | 1                     | 1                      | t044, t1109 | IV | IVC | 2 |
| A16                       | 1                     | 1                      | t044 | IV | IVC | 2 |
| A17                       | 1                     | 1                      | t044 | IV | IVC | 2 |

$^a$ Determined by the method of Oliveira and de Lencastre (34).

$^b$ Determined as described previously (28).

$^c$ Determined by previously described methods (17, 33).

$^d$ Number of different susceptibility profiles observed with the 11 antibiotics tested.
constituted the largest single cluster, with a generally increasing number of annually reported cases during the 12 years surveyed. In 2003 and 2004, the dramatic increase in the total number of MRSA cases (Table 1) was, however, primarily due to a single large hospital outbreak caused by a CC22 clone (51 and 142 cases, respectively), in addition to a general increase among other MRSA lineages, including CC80-MRSA-IV. The increase in the number of persons with CC80-MRSA-IV infection does not appear to be an artifact due to enhanced screening efforts, since the proportion found by screening was rather stable from 1999 to 2004, constituting approximately 15%.

**CA-MRSA.** We found 133/222 (60%) domestic infections to be CA infections. Thereby, domestic spread accounted for the majority of new cases, and transmission between household members was the most frequent source of transmission. The ability of CC80-MRSA-IV to disseminate in the community has previously been documented for households, kindergartens, work places, and sports teams (15, 16, 42), which correlates well with our findings. Patients infected outside hospitals are often children, adolescents, and their parents. Furthermore, CC80-MRSA-IV is observed as a CA pathogen more often than are MRSA isolates with other genetic backgrounds, since CA infections were encountered in only 27% of non-CA infections. Thereby, domestic spread accounted for the success of an active search-and-destroy policy. Multiple introductions of European CA-MRSA isolates into Denmark therefore seem likely.

**HA-MRSA.** Although CC80-MRSA-IV was predominantly a CA pathogen, 68 cases were designated HA infections, among which 18 were found in hospitals and another 50 were HACO infections. Thus, 68/244 (27.9%) isolates had some relationship to a hospital setting, indicating that the European CA-MRSA isolates have entered hospitals on several occasions. Interestingly, direct nosocomial transmission could be documented in only one case.

Because admission screening is usually not performed in Denmark, a likely scenario is that most of these patients were colonized prior to hospital admission, with infection developing at a later time.

In support of this, hospitalized patients infected with CC80-MRSA-IV were younger than patients infected with other MRSA strains.

Nosocomial spread of European CA-MRSA isolates has been reported only sparsely, with one case in Greece in 2001 and eight cases in Germany in 2005 (1, 2, 24). The absence of major nosocomial outbreaks caused by CC80-MRSA-IV is in contrast to reports regarding the USA300 MRSA clone in the United States (21, 37), which may indicate that CC80-MRSA-IV isolates are less well adapted to be sustained in hospital environments.

**Import.** We found a large proportion of patients reporting recent travel or family relationships to the Mediterranean area as well as to the Balkans (Serbia) and the Middle East. The Middle East and Mediterranean countries have also previously been proposed as a potential origin for European CA-MRSA strains isolated in Germany, Belgium, Switzerland, and France (6, 7, 13, 25).

Based on the scattered geographic distribution of the isolates in Denmark, dissemination seems to have occurred by introduction followed by a rather limited spread to household members and other close contacts. Eradication of the infections and carrier stage has been documented (8, 42), emphasizing the success of an active search-and-destroy policy. Multiple introductions of European CA-MRSA isolates into Denmark therefore seem likely.

**Types of infection.** SSTIs were encountered as the predominant type of infection (86.4%), which is in accordance with the findings of previous studies (8, 43). SSTIs caused by CA-MRSA have often been associated with the capability of expressing the PVL toxin (10, 23), and the genes encoding PVL were detected in all isolates examined in this study. However, the role of PVL in initiating SSTIs remains controversial, based on recent studies comparing the virulence of wild-type and isogenic PVL knockout isolates (22, 44). No other exo- or enterotoxin genes proposed to be involved in SSTIs were present in the tested isolates, except for etd, which has also been detected in German and French ST80-MRSA-IV isolates (29, 41). Most of the SSTIs were superficial infections, but invasive infections, including respiratory tract infections and bacteremia, were also found.

**Molecular typing and subtyping.** Subtyping of the European CA-MRSA isolates showed a high degree of diversity, distinguishing 17 different PFGE patterns with six different spa types and two different STs. Increasing diversity in PFGE patterns was encountered over time, which could be due to both genetic drift and the introduction of new variants. The high degree of molecular diversity makes it difficult to maintain the picture of “the European CA-MRSA clone” as a uniform clone, and it therefore seems more appropriate to designate the isolates as CC80-MRSA-IV isolates, according to the relation to CC80 and the carriage of SCCmec type IV. Current MLST nomenclature would place nine different STs (STs 80, 153, 397, 578, 66, 111, 119, 21, 37).
SCCmec typing. Characterization of SCCmec types support the earlier findings of SCCmec type IVc in the European CA-MRSA clone (5, 46), although this study indicates that several introductions of the SCCmec cassette have occurred. The isolates studied carried at least four different variants of the SCCmec IVc cassette (a to d). By Oliveira multiplex PCR, isolates carrying type IV or NT variants were obtained, and they contained either the recombination gene alleles ccrA2 and ccrB2 (variants a and b) or variant alleles from which no amplification products were obtained using the ccrA-, ccrB-, or ccrC-specific primers (variants c and d) (Table 2).

To further investigate the theory of multiple CC80-MRSA-IV introductions into Denmark, dru typing, which was previously successful in elucidating various subtypes of apparently clonal ST22 MRSA strains in Scotland and ST45 MRSA strains in Germany, was performed on a diverse subset of 20 strains (11, 48). However, only a single dru type (10a) was found. Interestingly, the same lack of diversity in dru has been observed for the USA300 CA-MRSA clone in the United States (40), which indicates that the degree of diversity in the dru segment may depend on the genetic lineage studied.

Antimicrobial susceptibility testing. Although the TSKF profile in addition to beta-lactam resistance was the predominant resistance profile, variation in antibiotic susceptibility within the CC80-MRSA-IV isolates was far more diverse than previously reported (46). Most notably, we found only 22 (7.7%) isolates to be resistant to fluoroquinolones, which likely constitutes a single clone, as deduced from their isolation primarily in 2001 and in a single county. In contrast, Witte et al. reported all ST80-MRSA-IV isolates to be resistant to ciprofloxacin (46). Furthermore, we found 17 (5.9%) isolates susceptible to fusidic acid, in contrast to previous German and French studies that found all ST80 isolates to be fusidic acid resistant (41, 46). Eleven isolates lacked the fusB gene and were tetracycline susceptible, which is likely due to the lack of a recently described plasmid, designated pUB102, that carries both the fusB and tetK genes (30).

Two additional isolates carried the fusB gene and were tetracycline resistant, suggesting that they carried pUB102 but did not express the fusidic acid resistance gene. Since travel and family relations to Mediterranean countries, the Balkans (Serbia), and the Middle East appear to be risk factors, special attention to these indicators could be prudent to diminish further introduction and dissemination. Transmission between household members seems to be the major route of spread, emphasizing the importance of including patient contacts when initiating eradication procedures. CC80-MRSA-IV has entered Danish hospitals on several occasions but has not caused any nosocomial outbreaks.

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