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Epidemiology of Emerging Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Denmark: a Nationwide Study in a Country with Low Prevalence of MRSA Infection

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Strict infection control measures introduced during the 1970s have kept the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections extremely low in Denmark. Nevertheless, similarly to other countries, MRSA infections began to appear in the community in the late 1990s. A nationwide surveillance program has collected and stored all MRSA isolates since 1988 and, since 1999, clinical information has been also recorded. We used this information and isolates in a detailed epidemiological and molecular analysis of the 81 MRSA infections identified in Denmark in 2001. MRSA isolates were characterized by pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing, and SCC*mec* typing. Comparison of the 45 community-onset MRSA (CO-MRSA) infections with the 36 hospital-acquired MRSA (HA-MRSA) infections showed several striking contrasts. Most CO-MRSA were recovered from skin and soft tissue infections caused by isolates carrying the Panton-Valentine leucocidin toxin genes, and the majority (84%) of isolates belonged to a single clonal type, ST80-IV, which has been found in the community in other European countries. Clone ST80-IV could be traced in Denmark back to 1993. ST80-IV was rarely found in HA-MRSA infections, which belonged to a large number of clonal types, including some pandemic MRSA clones. The low number of HA-MRSA infections and the diversity of MRSA clones in Danish hospitals may be the result of successful infection control measures that prevent spread of clones in hospitals. The mechanism of spread of the ST80-IV clone in the Danish community is not known, and new control measures are needed to control further spread of this and other CA-MRSA clones.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has remained a major cause of nosocomial disease worldwide (13, 14), causing 50% or more of hospital-acquired *S. aureus* infections in several countries (2). In addition, recent reports indicate that the epidemiology of MRSA may be undergoing a change through the emergence of community-acquired MRSA (CA-MRSA) (8, 19, 24, 34, 37). CA-MRSA is capable of causing infections in otherwise healthy people and may have a serious or even fatal outcome (4, 17).

In Denmark, a national surveillance system of staphylococcal disease has been in operation at the Statens Serum Institut since the late 1950s. As part of this program, all *S. aureus* isolates from blood cultures have been preserved since 1959, and from 1988 all MRSA isolates from both infection and colonization cases have also been collected and stored. In addition, beginning with 1999, clinical data have also been obtained. By the mid-1970s the incidence of hospital-acquired MRSA (HA-MRSA) was successfully reduced and has been kept at low levels since then due to the implementation of strict infection control measures and low consumption of antibiotics.

Recently, the National Surveillance System has identified an increase in the number of MRSA cases in Denmark. The low endemicity of MRSA and the availability of all MRSA isolates, as well as clinical and demographic information, provided an excellent opportunity to examine the epidemiological and clinical background of the increasing number of MRSA infections in the unique setting of Denmark, in an era in which reports from several countries have described the emergence of MRSA outside the usual hospital environment. Therefore, the purpose of the studies described here was to perform a detailed epidemiological analysis on each one of the 81 MRSA infections identified by the Danish surveillance system in 2001 and characterize the isolates by a variety of molecular typing techniques.

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MATERIALS AND METHODS

Clinical isolates and definitions. In 2001, a total of 105 MRSA isolates were referred to the Staphylococcus Laboratory at the Statens Serum Institut. The hospital discharge letter or the general practitioner's file was retrospectively requested for each patient. The following clinical data were recorded: reason for specimen collection (infection or active screening), place of infection acquisition (community-onset, hospital-acquired, or imported hospital-acquired infection), risk factor for acquisition of MRSA, and infection body site (skin and soft tissue, blood or central line, respiratory tract, postoperative wound, bone or joint, or urinary tract). Twenty-two of the isolates had been collected by active screening and were not investigated further. Two isolates could not be subcultured, leaving a total of 81 MRSA isolates from infections available for this study.

Due to the finding of a major clone among the MRSA isolates from 2001 (see Results), we searched for this clone from 1988 onwards in the MRSA collection from the Statens Serum Institut. We screened isolates for concomitant resistance to kanamycin and susceptibility to gentamicin—a characteristic of this clone. These isolates were typed by pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST), and SCC*mec* typing. Seven additional iso-

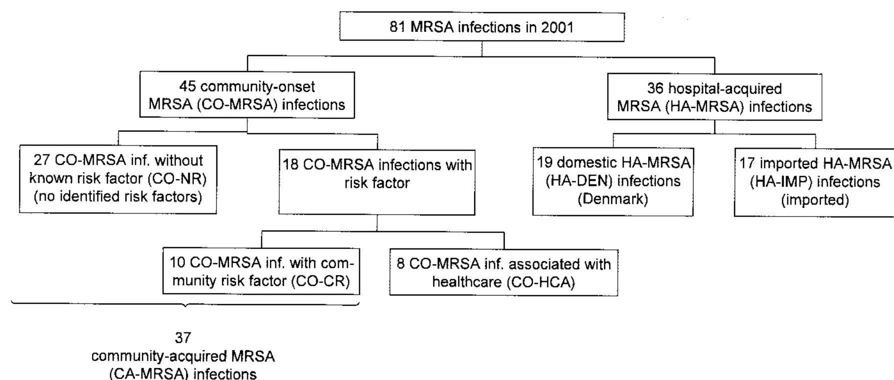

FIG. 1. Classification of 81 MRSA infection isolates from Denmark in 2001.

lates with a similar PFGE pattern were obtained from other European countries (Greece, Finland, France, and The Netherlands) for comparison purposes.

For the classification of infection acquisition, we used the modification recently proposed by Salgado et al. for MRSA infections (33), adding a further category for community-onset MRSA (CO-MRSA) with identified community risk factors, thus resulting in the following five possible types of MRSA infections: (i) domestic, HA-MRSA for infections acquired during hospitalization in Denmark (HA-DEN); (ii) imported HA-MRSA for infections acquired after contact with a healthcare facility outside Denmark (HA-IMP), typically by transfer of an MRSA-infected patient from a foreign hospital; (iii) CO-MRSA infections with no identified risk factors (CO-NR); (iv) CO-MRSA infections with an identifiable community risk factor (CO-CR), such as being a household member or in close contact with a patient with CO-MRSA or recent travel to a country with high prevalence of HA-MRSA with no known contact with healthcare in that country; (v) CO-MRSA infections with identified healthcare risk factors (CO-HCA), such as previous MRSA colonization, recent hospitalization (<12 months), regular ambulatory contact (e.g., dialysis patient), or being a household member or in close contact with a patient with HA-MRSA. CO-NR and CO-CR were considered "true" community-acquired MRSA (CA-MRSA).

Susceptibility tests. Susceptibility tests for penicillin, methicillin, streptomycin, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline, fusidic acid, rifampin, and ciprofloxacin were performed using Neosensitabs (Rosco, Taastrup, Denmark) on Danish blood agar (Statens Serum Institut, Copenhagen, Denmark) using a semiconfluent inoculum and overnight incubation at 35 to 36°C, according to the manufacturers' instructions and breakpoints (7, 32). Isolates were also screened for MICs of oxacillin with the E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. *S. aureus* ATCC 29213 was included in all runs for quality control.

PFGE. All isolates were subjected to PFGE analysis, which was performed according to the HARMONY protocol (28), and analyzed using the Bionumerics version 2.5 software (Applied Maths, Kortrijk, Belgium). Concatamerized phage λ DNA (New England Biolabs) was included in every sixth lane as a molecular weight standard to normalize distances within each gel, whereas SmaI-digested *S. aureus* NCTC 8325 was used to normalize distances among the gels. Only DNA fragments of sizes in the range of the λ ladder (50 to 1,000 kb) were included in the analysis. PFGE patterns were identified on a unweighted pair group method with averages dendrogram based on Dice coefficients, where optimization and band position tolerance were set at 1.0 and 2.3%, respectively. A similarity coefficient of 80% was selected to define the patterns (27).

Multiplex PCR SCC*mec* typing and *ccr* typing. SCC*mec* types were determined by a multiplex PCR strategy which generated a specific amplification pattern for each structural type of SCC*mec* (30), and the types were further characterized by *ccrAB* typing as previously described by Okuma et al. (29). According to Okuma and colleagues, presumptive SCC*mec* typing may be performed by simply combining the information for the *ccrAB* allele (*ccrAB* typing) with the genetic organization of the *mecA* regulatory region (*mecA* complex). Since the SCC*mec* multiplex strategy provides information on the *mecA* complex structure (presence or absence of *mecI*), combining this strategy with *ccrAB* typing introduced an extra degree of confidence in the presumptive assignment of SCC*mec* types. This is particularly relevant for SCC*mec* type IV, the dominant type among the collection studied, which is a heterogeneous cassette and is identified by the presence of just two bands by the multiplex PCR strategy. SCC*mec* type IV isolates that contain an insertion of pUB110 downstream of *mecA* have been previously described as IV-A (30). However, to avoid confusion with the established nomenclature of IVa, IVb, and IVc (21), we refer to SCC*mec* IV isolates containing pUB110 as SCC*mec* IV variants. Isolates positive only for the control primers of the *ccrAB* locus were classified as nontypeable for *ccrAB*. Isolates negative for all primer sets, including the control primers, were classified as *ccrAB* negative after verification by Southern blotting analysis with a DNA probe specific for the *ccrAB* locus. Due to the recent description of SCC*mec* type V carrying the *ccrC* allele (20), isolates classified as nontypeable or negative for *ccrAB* were tested for the presence of the *ccrC* allele, as previously described (20).

Southern blotting. DNA transfer to Hybond N^+ membranes (Amersham Pharmacia Biotech Inc., Piscataway, N.J.) was performed as previously described (10). Southern hybridization was performed with the ECL Direct Prime labeling and detection systems (Amersham Pharmacia Biotech Inc.) according to the manufacturer's instructions. The DNA probe for the *ccrAB* locus was obtained by PCR amplification of a positive isolate with primers αC and βC (29).

Detection of PVL genes. Detection of Panton-Valentine leukocidin (PVL) genes was performed by PCR as previously described (12).

***spa* typing and MLST.** *spa* typing and MLST were performed as previously described (9, 15, 23, 35). Sequences of both strands were determined at Macrogen (Seoul, South Korea), and sequence analysis was carried out using the DNAStar software (Lasergene, Madison, Wis.). The new *spa* type assignments were provided by B. N. Kreiswirth and S. Naidich (personal communication), and MLST sequence types (ST) were assigned through the MLST database (http://www.mlst.net).

RESULTS

Epidemiological analysis. The 81 MRSA infection isolates from Denmark in 2001 were classified into five groups (Fig. 1). Thirty-six isolates (44%) were HA-MRSA, either in Denmark (HA-DEN, $n = 19$) or during hospitalization abroad (HA-IMP, $n = 17$). Forty-five isolates (56%) were from CO-MRSA infections with either no identified risk factor (CO-NR, $n = 27$) or with risk factors ($n = 18$). These were further divided into CO-MRSA with healthcare risk factors (CO-HCA, $n = 8$), and CO-MRSA with community risk factors (CO-CR, $n = 10$). In this CO-CR group, the risk factor for 4 of the 10 patients was recent travel to countries with high endemicity of MRSA but without known contact with healthcare while abroad.

CO-MRSA infections with no identified risk factors (CO-NR) and CO-MRSA with a community risk factor (CO-CR) can be considered to represent true CA-MRSA infections, and these accounted for 46% (37 out of 81) of all MRSA infections in Denmark during 2001. CA-MRSA isolates were predominantly associated with skin or soft tissue infections (32 out of 37; 86%) and younger patients (Table 1).

CO-MRSA isolates with health care risk factors (CO-HCA)

TABLE 1. Distribution of MRSA infection isolates by age of patient, PFGE pattern, and infection body site

Parameter	CO-MRSA			HA-MRSA		Total
	CA-MRSA		CO-HCA	HA-DEN	HA-IMP	
	CO-NR	CO-CR				
n	27	10	8	19	17	81
Median age (range) (yrs)	32 (1–88)	29 (5–53)	47 (0–73)	56 (25–79)	40 (6–72)	
PFGE pattern, by infection body site						
Skin or soft tissue	19 A, 4 non-A	8 A, 1 non-A	2 A, 4 non-A		4 A, 4 non-A	46
Postoperative wound		1 non-A		2 A, 7 non-A	4 non-A	14
Respiratory tract[a]	3 A		1 A, 1 non-A	1 non-A	2 non-A	8
Blood or central line	1 A			4 non-A	2 non-A	7
Bone or joint[b]				4 non-A	1 non-A	5
Urinary tract				1 non-A		1
Total	23 A, 4 non-A	8 A, 2 non-A	3 A, 5 non-A	2 A, 17 non-A	4 A, 13 non-A	81

[a] Ear (4), lung (2), conjunctiva (2), sinus (1).
[b] Bone (2), joint (3).

were also generally associated with skin or soft tissue infections in patients with frequent hospital outpatient contacts. HA-MRSA isolates, whether acquired in Denmark or abroad, were mostly associated with postoperative wound infections, septicemia, and severe bone or joint infections (Table 1).

Phenotypic and genotypic characterization of MRSA isolates. Each of the 81 MRSA infection isolates—36 HA-MRSA and 45 CO-MRSA—was analyzed by PFGE. We identified 13 different PFGE patterns: 10 of the patterns (A, H, L, J, O, Q, R, S, T, and V) included two or more isolates and were labeled with capital letters and with a number suffix to indicate subtype; three additional patterns were represented by single isolates only (YA, YB, and YC). The largest cluster, representing 40 of the 81 isolates, was PFGE type A, which could be divided into five subtypes: A1 ($n = 34$), A2 ($n = 2$), A3 ($n = 1$), A4 ($n = 2$), and A5 ($n = 1$). Pattern A isolates were found in all parts of Denmark and were often associated with small local community outbreaks (Fig. 2). Irrespective of their origin, the PFGE pattern A isolates were generally resistant to streptomycin (93%), kanamycin (95%), tetracycline (93%), and fusidic acid (93%) and susceptible to gentamicin (0% resistant), erythromycin (2.5% resistant), clindamycin (0% resistant), rifampin (5% resistant), and ciprofloxacin (0% resistant). Among non-pattern A isolates, HA-MRSA and CO-HCA MRSA isolates were significantly more often multiresistant, i.e., resistant to at least three classes of antibiotics, than CA-MRSA isolates ($P < 0.001$, chi-square test) (data not shown).

Most CO-MRSA isolates had low-level oxacillin resistance (MIC range, 8 to 64 μg/ml) compared with most HA-MRSA isolates, which had oxacillin MICs higher than 256 μg/ml (data not shown).

PFGE type A isolates were significantly more frequent among CO-MRSA than HA-MRSA isolates (84 versus 18%; $P < 0.001$, chi-square test) (Table 1). Among CO-MRSA isolates, PFGE pattern A was significantly more frequent among CA-MRSA than CO-HCA MRSA (84 versus 38%; $P < 0.014$, Fisher exact test) (Table 1).

MLST, *spa* typing, and SCC*mec* typing. MLST and *spa* typing were performed on 36 of the 81 MRSA isolates from 2001, which included representatives of each of the 13 PFGE types as well as several PFGE subtypes identified among the 81 MRSA isolates. In order to test the homogeneity of the largest cluster, PFGE type A, a total of nine PFGE isolates representing each of the five subtypes and originating from both CO-MRSA and HA-MRSA infections were included among the isolates typed for *spa* and MLST pattern. Results are shown in Table 2, which summarizes the genetic portraits of CO-MRSA and HA-MRSA isolates.

The dominant clone, defined by PFGE pattern A and its

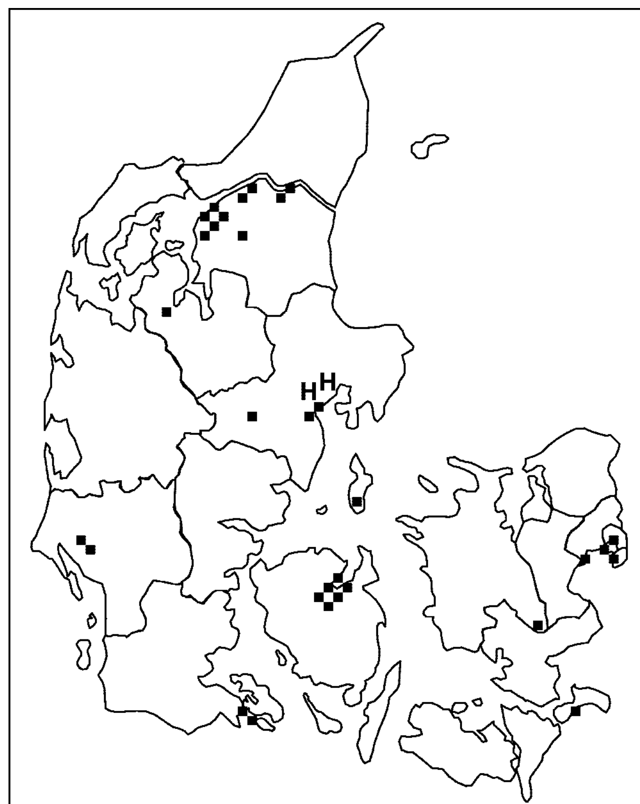

FIG. 2. Geographic distribution of clone ST80-IV in Denmark in 2001. One case from the island of Bornholm is not marked. H, hospital-acquired MRSA.

TABLE 2. Characterization of CO- and HA-MRSA isolates

PFGE type	No. in collection	*spa* typing		MLST		SCC*mec* typing[b]			PVL
		Profile[a]	Type	Allelic profile	ST	Multiplex PCR	*ccr* typing	SCC*mec* type	
CO-MRSA (n = 45)									
A_1, A_3, A_4, A_5	33	UJGBBPB (1)	70	1-3-1-14-11-51-10	80	IV	2	IV	+
A_2	1	UJGBBPB (1)	70			IV	Negative	NT[c]	+
H_3	1	TMDMGMMK	437	1-4-1-4-12-25-10	225	II	2	II	−
H_6	1								
L_1	1								
L_2	2	XKAKAOMQ (35)	19	2-2-2-2-6-3-2	30	IV	2	IV	+
O_1	1	ZDMA3KB	434	19-23-15-2-19-20-42	375	IV	2	IV	−
Q_1	1	YHFGFMBQBLO (30)	4	3-3-1-12-4-4-16	247	I	1	I	−
R_1	1	UJ2GMKKPNSG	207	46-75-49-44-13-68-60	152	NT	C	NT	+
T_1	1	XKAKEEMBKB	374	10-14-8-6-10-3-2	45	NT	C	NT	−
T_2	1								
V_2	1	TJEJNF2MNF2MOMOKR	436	7-6-1-5-8-8-6	22	IV	2	IV	−
HA-MRSA (n = 36)									
A_1	5	UJGBBPB (1)	70	1-3-1-14-11-51-10	80	IV	2	IV	+
A_2	1	UJGBBPB (1)	70			IV	Negative	NT	+
H_1	1	TJMBMDMJMK	2	1-4-1-4-12-1-10	5	IV	2	IV	−
H_2	1	TJMBDMGMK (30)	45			IV	2	IV	−
H_4	1	TJMBDMGMK (30)	45			NT	2	IV	+
H_5	1	TJMEMDMGMK (35)	24	1-4-1-4-12-1-10	5	II	2	II	−
H_2	2	TO2MBMDMGMK	385	1-4-1-4-12-24-29	228	I	1	I	−
J_1	1	WGKAOMQ (35)	3	2-3-1-1-4-4-3	239	NT	Negative	NT	−
J_2	1	WGKAOMQ (35)	3			NT	3	III variant	−
J_3	1	WGKAQQ (3)	351	58-3-1-1-4-4-3	444	NT	3	III variant	−
J_4	1								
J_5	1								
J_6	1								
L_1	2	WGKAKAOMQQQ (35)	16	2-2-2-2-6-3-2	30	NT	2	IV	−
L_2	2	XKAKAOM (35)	30	2-2-2-2-6-3-2	30	IV	2	IV	+
O_2	1								
Q_1	1	YHFGFMBQBLO (35)	4	3-3-1-12-4-4-16	247	I	1	I	−
Q_2	1	YGFMBQBLO (3)	363	3-3-1-1-4-4-3	8	NT	2	IV	−
Q_4	1								
R_2	1								
S_1	2	YHGFMBQBLO (35)	1	3-3-1-1-4-4-3	8	IVA	2	IV variant	−
S_2	1	YHGFMBQBLO (35)	1			IVA	2	IV variant	−
S_3	1	YHGCMBQBLO (35)	7	3-3-1-1-4-4-3	8	IVA	2	IV variant	−
T_2	1								
V_1	1								
YA	1	Y2EJCLMBPB	480	1-26-28-18-18-33-27	509	I	1	I	−
YB	1	WGKAOMQ (35)	3			NT	3	III variant	−
YC	1	WGKAOMQ (35)	3			III	3	III	−

[a] Values in parentheses indicate the original reference for the *spa* profile.
[b] As described in Material and Methods.
[c] NT, nontypeable.

variants, was characterized by *spa* type 70 (UJGBBPB), ST 80, and SCC*mec* type IV (clone ST80-IV). All of these isolates were PVL positive. Thirty-four of the 45 (76%) CO-MRSA isolates had this genetic background. The remaining 11 CO-MRSA isolates belonged to seven different clonal types: two were PVL positive and five were PVL negative (Table 2).

Only six of the 36 (17%) HA-MRSA isolates showed the typical genetic background of clone ST80-IV. The remaining 30 isolates belonged to eight different PFGE types (Table 2). With the exception of isolates of clone ST80-IV and some isolates belonging to clone ST30-IV, the remaining HA-MRSA isolates were negative for PVL.

Results of SCC*mec* typing are summarized in Table 2. In addition to the typical SCC*mec* types I to IV that were identified by the combination of multiplex PCR and *ccrAB* typing, we found variants of type III and four isolates that could not be classified by multiplex PCR and were negative for *ccrAB*. Two of the *ccrAB*-negative isolates contained *ccrC*, which was recently described by Ito and colleagues (20), in SCC*mec* type V. However, the definitive assignment of the SCC*mec* type in these two isolates requires further investigation, namely, the characterization of the *mec* complex, since SCC*mec* type III isolates carry in the *mecA* downstream vicinity a region highly homologous to *ccrC* (20).

Molecular typing identified several isolates that were identical or closely related to previously described epidemic MRSA

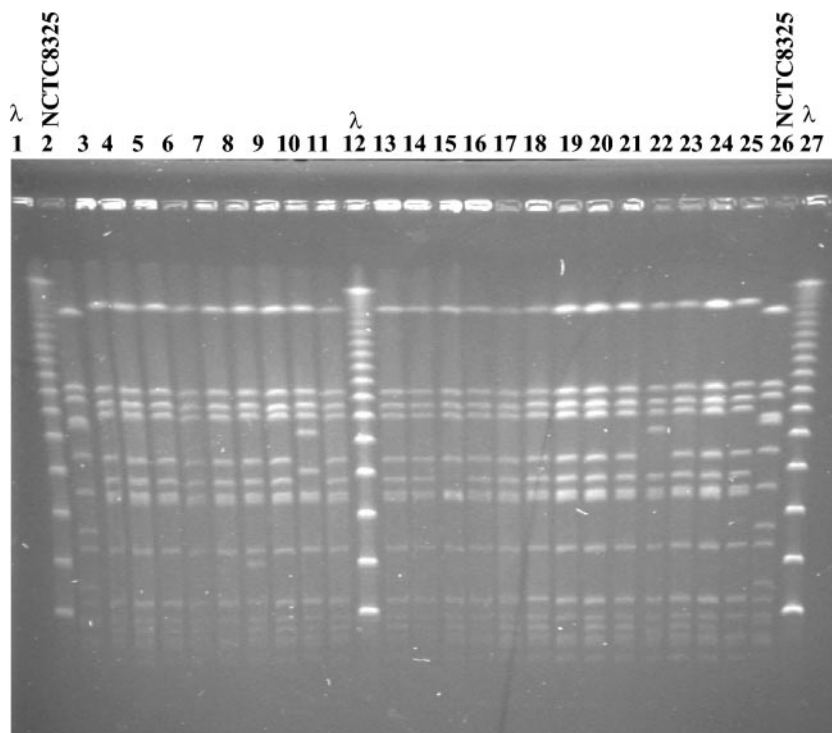

FIG. 3. PFGE of SmaI macrorestriction fragments of representative isolates from the retrospective detection of clone ST80-IV compared with ST80-IV isolates from other countries. Lanes 1, 12, and, 27, molecular size standards (bacteriophage lambda oligomers); lanes 2 and 26, NCTC 8325; lane 3, DEN24329/93; lane 4, DEN1451/94; lane 5, DEN1452/94; lane 6, DEN18851/95; lane 7, DEN22940/95; lane 8, DEN4250/96; lane 9, DEN19957/96; lane 10, DEN11819/97; lane 11, DEN15779/97; lane 13, DEN23612/98; lane 14, DEN16143/98; lane 15, DEN16915/99; lane 16, DEN17475/99; lane 17, DEN2949/01; lane 18, DEN4199/01; lanes 19 and 20, GRE14 and GRE143, respectively (1); lane 21, E31 (34); lanes 22 and 23, HT0401 and HT0490, respectively (37); lanes 24 and 25, 1418/02 and 27/03 (Heck, unpublished).

clones. These included the Archaic clone (ST247-I, *spa* type 4, YHFGFMBQBLO); the Pediatric clone (ST5-IV, *spa* type 24, TJMBMDMGMK); the New York/Japan clone (ST5-II, *spa* type 24, TJMBMDMGMK); the Brazilian/Hungarian clone (ST239-III, *spa* type 3, WGKAOMQ); EMRSA 15 (ST22-IV, *spa* type 436, TJEJNF2MNF2MOMOKR); clone V/USA (ST8-IV, *spa* type 1, YHGFMBQBLO); and Southern Germany EMRSA-1 (ST228-I, *spa* type 385, TO2MBMDMGMK) (16, 31). Interestingly, most of these clonal types were recovered from HA-MRSA infections.

Retrospective detection of clone ST80-IV and comparison with European isolates. Isolates with PFGE pattern A have been recognized in Denmark since late 1997, when four infections due to MRSA with this PFGE pattern were observed. Sixteen more cases of PFGE pattern A-related MRSA infections were observed in 1998, followed by 22 cases in 1999 and 20 cases in 2000. During the same period of 1997 to 2000, the total number of MRSA isolates each year (including colonizing isolates found by active screening) was 41, 54, 67, and 97, respectively. The collection of MRSA isolates at the Statens Serum Institut was screened from 1988 onward for the presence of clone ST80-IV based on the clone's characteristic susceptibility pattern, i.e., resistance to kanamycin and susceptibility to gentamicin, followed by PFGE confirmation. Clone ST80-IV was not detected prior to 1993. Two isolates were found in 1993, four in 1994, three in 1995, and three in 1996. Twenty-two PFGE pattern A isolates from 1993 to 1999 were further characterized. All isolates had *spa* type 70 (UJG BBPB), SCC*mec* type IV, and PVL genes. By *ccrAB* typing, 20 isolates were found to belong to type 2, one was nontypeable, and one was *ccrAB* negative. By MLST 20 were ST80, whereas the remaining two represented a new single-locus variant (SLV) of ST80 (ST153).

Finally, we obtained presumptive ST80-IV MRSA isolates from colleagues in Finland (34), Greece (1), France (37), and The Netherlands (M. Heck, unpublished results). All these isolates were confirmed to belong to clone ST80-IV by *spa* typing, MLST, and SCC*mec* typing and by the presence of PVL genes. Figure 3 shows the PFGE profiles of isolates belonging to clone ST80-IV isolated in Denmark in 1993 to 2001 and in Finland, Greece, France, and The Netherlands.

DISCUSSION

In this study, CA-MRSA infections were defined as infections acquired in the community associated with no identified risk factors (CO-NR) or with identified community risk factors (CO-CR). CO-MRSA isolates associated with identified healthcare risk factors (CO-HCA) were not included in the CA-MRSA cluster (see detailed definitions in Materials and Methods).

Using such stringent criteria, the most interesting feature of this study is that it provides a striking demonstration of the emergence of true CA-MRSA infections in a country that has

an established record of success in keeping the frequency of MRSA infections at a very low and steady level. As a matter of fact, due to the introduction of strict infection control measures and the low consumption of antibiotics in Denmark, the prevalence of MRSA in *S. aureus* bacteremia declined sharply from approximately 20% at the end of the 1960s to less than 1% throughout the 1980s and 1990s (http://www.ssi.dk/graphics/dk/overvagning/Annual02.pdf). During the same 20-year period, the proportion of *S. aureus* bacteremias that were hospital acquired decreased from 63 to 54%, while community-acquired *S. aureus* bacteremias increased from 23 to 32% (unpublished data from the Staphylococcus Laboratory, Statens Serum Institut).

Not only was it unusual that the majority of the MRSA infections in this study occurred outside hospitals, but also that the infection sites involved and the properties of bacterial isolates causing them were unusual. The overwhelming majority of CO-MRSA infections were skin and soft tissue infections, whereas HA-MRSA infections were primarily postoperative wound, respiratory tract, or bloodstream infections (Table 1). The genetic background of CO-MRSA infections was also unique. The majority of CO-MRSA infections (34 out of 45) were associated with one single clone (PFGE type A, clone ST80-IV). Clone ST80-IV is not unique to Denmark, and it has been identified in Greece (1), France (12), Switzerland (25), Finland (34), Germany (38), Belgium (11), Norway (36), Scotland (5), Sweden (B. Olsson-Liljequist, unpublished results), and The Netherlands (Heck, unpublished results). While in most cases this clone has been associated with CA-MRSA infections, it was first isolated in 1998 from a hospitalized patient in Greece (1).

The remaining Danish CO-MRSA isolates were predominantly closely related to other CA-MRSA clones previously described in Europe, the United States, and Australia (37), such as clones ST8-IV, ST22-IV, ST30-IV, and ST45-IV (Table 2). By contrast, HA-MRSA isolates were predominantly closely related to previously described pandemic MRSA clones (2, 16, 31), namely, to the Archaic/Iberic group (ST247-I and ST8-I) and the Brazilian clone (ST239-III), both belonging to a clonal complex (CC8) and several lineages included in the clonal complex (CC 5), ST5-II and ST228-I (Table 2).

PVL genes were detected in all ST80-IV isolates, in two of the ST30-IV isolates, one of the ST152-NT isolates, and also in an isolate that seems to be related to ST5 on the basis of *spa* typing (*spa* type 45) (Table 2). The PVL genes encode a cytotoxin associated with skin and soft tissue infections and also, as recently described, associated with severe necrotizing pneumonia with high mortality rates (4, 26). The fact that many CO-MRSA isolates were PVL positive provides the most likely explanation for the predominance of skin and soft tissue infections found among Danish CO-MRSA infections.

As in many countries, systematic screening of patients for MRSA carriage at hospital admission is not performed in Denmark. The sporadic PVL-positive isolates classified as HA-MRSA, which included isolates imported from foreign hospitals, could therefore have been acquired in the community before hospital admission. Nevertheless, the risk of emergence of PVL-positive isolates in existing HA-MRSA clones is an issue of serious concern and could result in the emergence of multidrug-resistant HA-MRSA isolates with increased virulence (12, 37).

As the Staphylococcus Laboratory of the Statens Serum Institut has kept all Danish MRSA isolates since 1988, we were able to trace ST80-IV as far back as 1993. The origin of this index case of clone ST80-IV identified in Denmark in 1993 is not known. The retrospective search identified another isolate from 1993 with a new SLV type of ST80 (ST153). After sporadic appearances between 1993 and the end of 1997, clone ST80-IV appeared in Denmark with increased incidence from 1997 through 2001. Preliminary results for 2002 and 2003 suggest that the incidence of ST80-IV in Denmark has now stabilized at the 2001 level and continues to be true CA-MRSA associated with skin and soft tissue infections (unpublished data from the Staphylococcus Laboratory, Statens Serum Institut).

The clinical data available for the CA-MRSA clonal types ST30-IV and ST152-NT provide information on the possible geographical origins of these isolates. One of the ST30 MRSAs was isolated from a person who had traveled to the Philippines, and the other ST30 isolate was recovered from a Danish seaman. The clonal type ST30-IV, which is PVL positive, has been previously described among CA-MRSA from Oceana (29, 37). The MRSA isolate with the previously unknown ST152 and characterized by a new *spa* type 207 (UJ2GMKKPNSG) was recovered from a Danish patient who had been hospitalized in the Kosovo region (Serbia, Montenegro). Interestingly, ST152 is an SLV of ST377, recently identified in an MRSA isolate from The Netherlands (http://www.mlst.net).

The almost simultaneous and worldwide emergence of CA-MRSA isolates with SCC*mec* type IV and PVL genes suggests that these lineages are particularly fitted to become successful community-based pathogens (12, 37). CA-MRSA clones may have emerged as the result of successive genetic events that involve the acquisition of PVL genes and the type IV SCC*mec* (or other small SCC*mec* cassettes, such as the recently described SCC*mec* type V [20]), most likely from coagulase-negative staphylococci indigenous to the skin flora, as previously proposed by others (6, 22).

In testing for the SCC*mec* types carried by MRSA isolates, we found several in which a specific SCC*mec* type could not be assigned either by the multiplex PCR strategy (30) or by PCR amplification of the *ccrAB* and *ccrC* alleles (20, 29), suggesting either the presence of new variants or a novel SCC*mec* type. The origin of these yet-uncharacterized SCC*mec* types could also be the methicillin-resistant coagulase-negative staphylococci ubiquitous to the skin flora (6, 22), as the majority of these isolates originated from skin and soft tissue infections. This possibility may be best tested in countries with a low prevalence for MRSA, such as Denmark. Similar findings were recently reported from Norway by Hanssen et al. (18).

In conclusion, MRSA is no longer confined to sporadic cases in hospitals in Denmark. The increase in CA-MRSA infections observed, even in countries with a low prevalence of HA-MRSA, such as Denmark, Sweden, Norway, and Finland, calls attention to the fact that the well-established infection control measures that have worked in hospitals may be less effective in controlling the public health threat represented by CA-MRSA.

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