

Molecular Epidemiology of an Outbreak Caused by Methicillin-Resistant *Staphylococcus aureus* in a Health Care Ward and Associated Nursing Home

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Our point-prevalence survey followed an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in a long-term care facility and identified five MRSA strains, of which two possessed an outbreak genotype not encountered previously and three had another profile. All of them possessed SCCmec type V. Six methicillin-sensitive *S. aureus* strains were genotypically related to the epidemic strains.

In long-term care facilities (LTCFs) methicillin-resistant *Staphylococcus aureus* (MRSA) strains are more often associated with colonization than clinical infection. LTCFs may also represent a reservoir of MRSA, and therefore create a two-way flow of MRSA between hospitals and LTCFs. Only a few studies have previously described the molecular epidemiology of MRSA in nursing homes (2, 4, 12, 15, 16). In all of them, MRSA strains have been identical or closely related to the strains found in hospitals in the region.

An outbreak of MRSA in a health care ward and an associated nursing home of a 5,000-inhabitant municipality in northern Finland during autumn 2003 was caused by an outbreak MRSA strain of a new pulsed-field gel electrophoresis (PFGE) (FIN-22) profile and a multilocus sequence type (MLST) (ST-27) not encountered previously in Finland. With this intriguing genotype knowledge and the epidemiological fact that the LTCF is located in a remote area with limited patient transfer, we performed a point-prevalence survey 6 months later. We also assessed the molecular epidemiology of MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) strains, comparing the results to the national strain collection.

Setting and outbreak. A 34-bed health care ward (HCW) mainly takes care of elderly patients with multiple underlying diseases but also provides primary care, and an associated 46-bed nursing home (NH) is only for the elderly. Patient transfer from HCW to the nearby secondary-care hospital occurs approximately 0 to 2 times per week, and from the NH to the HCW and to the secondary-care hospital approximately 40 and 2 to 5 times per year, respectively.

The first isolate of MRSA was found in a urine culture in the HCW in August 2003. The index patient was placed in a single room and cared for according to contact precautions. All three roommates were screened for MRSA. All in-patients of the HCW

and NH were screened twice between October and November in 2003 and once on 17 February 2004. The staff was not screened. A total of 255 screening specimens for MRSA were taken between August and December in 2003; 12 new patients with a screening specimen positive for MRSA were found, all from LTCFs. In total, 726 clinical bacterial cultures were examined in the whole municipality in 2003. None were positive for MRSA except the urine culture of the index patient. The new PFGE profile (FIN-22) was obtained from all 13 isolates from different patients.

Point-prevalence survey. On 26 February 2004, all in-patients who volunteered (76 of 80) had their nostrils and skin lesions swabbed, and catheter urine samples were cultured. The following data were collected for each patient: age, sex, use of antimicrobials, foreign devices, and length of nursing stay. The screening swabs (Proback transport swab; Schofield St-Heywood, United Kingdom) were cultivated on nonselective sheep blood agar (SBA), and on selective oxacillin resistance screening agar (ORSAB CM1008, Oxoid, United Kingdom) plates. The SBA plates were incubated for 48 h and ORSAB for 96 h and inspected daily. *S. aureus*-like colonies were picked and subcultured onto SBA. The colonies were identified by conventional biochemical tests (8).

Antimicrobial resistance to cefoxitin, cephalixin, gentamicin, tobramycin, erythromycin, clindamycin, chloramphenicol, ciprofloxacin, levofloxacin, rifampin, fusidic acid, trimethoprim-sulfamethoxazole, tetracycline, vancomycin, teicoplanin, linezolid, and mupirocin (Oxoid, Hampshire, England) was tested by the disk diffusion method. Resistance to methicillin was determined by the oxacillin MIC test (E-test; AB Biodisk, Solna, Sweden) according to the manufacturer's instruction. All biochemically identified *S. aureus* strains (one strain per specimen) were tested by both GenoType MRSA and GenoType *Staphylococcus* tests (Hain Lifesciences, Germany) to determine *mecA*- and *S. aureus*-specific fragments and the Pantone-Valentine leukocidin (PVL) gene, respectively. All genetically confirmed *S. aureus* isolates (one strain per specimen unless both *mecA*-positive and *mecA*-negative strains were found) were genotyped with PFGE (9) and interpreted as described elsewhere (14).

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TABLE 1. Characteristics of carrier groups at the time of the point-prevalence study

Parameter	MRSA carriers	MSSA carriers	<i>S. aureus</i> noncarriers
No. (%)	5 (7)	21 (28) ^a	52 (68)
No. of sites colonized (%)			
Nostril	4 (5)	20 (26)	52 (68)
Skin lesion	1 (10)	4 (40)	5 (50)
Urine	0 (0)	1 (25)	3 (75)
Median age, yr (range)	82 (75–94)	80 (35–93)	86 (50–99)
No. of males (%)	2 (40)	8 (38)	17 (33)
No. of given antibiotic treatment (%)	2 (40)	7 (33)	11 (21)
No. with urinary catheter (%)	0 (0)	1 (5)	3 (6)
Nursing period, median months (range)	6 (2–92)	6 (<1–57) ^b	7 (<1–71) ^b

^a Two of the methicillin-sensitive *Staphylococcus aureus* carriers were also colonized with methicillin-resistant *S. aureus*.

^b The data were missing for two MSSA carriers and nine non-*S. aureus* carriers.

The MLST of the outbreak index strain and one representative MRSA and MSSA strain with similar PFGE patterns found in this point-prevalence study was performed as described earlier (3). Sequences were compared to data in the MLST database (www.mlst.net) and the sequence type (ST)

was assigned. The SCCmec types were determined as described earlier (6, 7, 11).

A total of 90 specimens, 76 from nostrils, 10 skin lesions, and 4 catheter urine, were obtained from 76 in-patients (*n* = 32 in the HCW, *n* = 44 in the NH). Of the 90 specimens, 30 (33%) were positive for *S. aureus* and five of them (17%) were positive for MRSA. Of the 76 patients, 24 (32%) were *S. aureus* carriers and five (20%) of them were positive for MRSA, the prevalence of MRSA being 7% (Table 1). The demographics of the different carrier groups did not differ from each other (Table 1).

Two of the five MRSA isolates were from patients recorded positive during the outbreak in 2003 (one from the NH, and another from the HCW), one of the patients was found to be positive for MRSA for the first time 9 days before the point-prevalence study in February 2004 (from the NH), and two of the patients were new carriers (both from the NH). Of the remaining 11 patients who had previously been found positive for MRSA during the outbreak in 2003, five had died, one had been discharged, and five were now culture negative.

All five MRSA isolates were resistant only to beta-lactams (Fig. 1), expressing an oxacillin MIC of 2 to 32 mg/liter. All MSSA strains were sensitive to all antimicrobials tested except one strain, which was resistant to chloramphenicol.

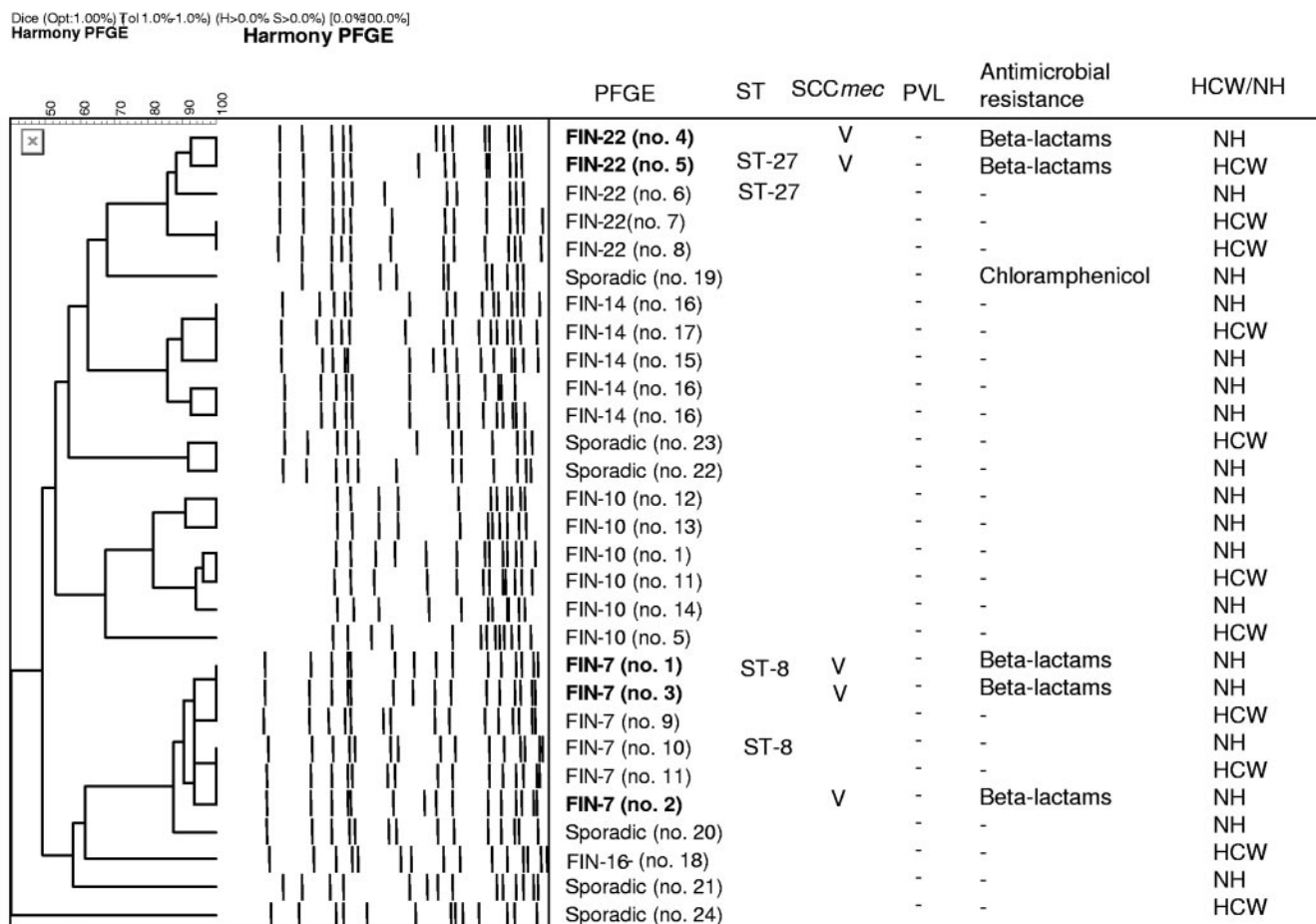


FIG. 1. Dendrogram based on the Dice coefficient of pattern similarity, obtained by SmaI macrorestriction analysis of *S. aureus* isolates. The MRSA strains are in bold. The patient numbers are in parentheses. HCW, health care ward; NH, nursing home.

Eleven different PFGE profiles were detected among a total of 30 *S. aureus* isolates and two different PFGE profiles among five MRSA isolates. Two isolates were indistinguishable or closely related to the outbreak FIN-22 PFGE profile (Fig. 1; patients 4 and 5), and three isolates showed a genotype of another Finnish epidemic, FIN-7 PFGE profile MRSA strain (Fig. 1; patients 1 to 3). Two representative strains of FIN-22 and FIN-7 (one MRSA and one MSSA) were characterized as ST-27 and ST-8 by MLST, respectively (Fig. 1; patients 5, 6, 1, and 10). All five MRSA strains possessed the F locus typical of SCCmec type III (414 bp) detected by multiplex PCR. The strains were, however, negative for recombinase genes *ccrA* and *ccrB* but possessed *ccrC* as detected by specific primers, thus belonging to SCCmec type V (Fig. 1). None of the 30 *S. aureus* strains (MRSA and MSSA) were positive for the PVL gene (Fig. 1).

The original MRSA outbreak consisting of isolates possessing a PFGE profile new to Finland and an internationally rare MLST type was confined within 6 months. However, three new MRSA cases related to another Finnish epidemic genetic profile (FIN-7 and ST-8) were detected. This strain has spread rather widely in Finland.

All the MRSA strains found in this survey possessed SCCmec type V, indicating that the same mobile genetic element may have integrated into the chromosome of the patient *S. aureus* strains. SCCmec type V has been reported to be associated with community acquisition and to be distributed among coagulase-negative staphylococcal species, especially *S. haemolyticus* (6, 10).

To our knowledge, this is the first study that compares the genotypes of MRSA and MSSA within a nursing home setting. Among the 25 MSSA and five MRSA strains, 11 different PFGE patterns obtained from 24 different persons indicate wide dissemination of different *S. aureus* genotypes in a small population of LTCFs studied. However, six of the MSSA strains from six different patients were related to MRSA genotypes found in this study, and altogether 18 of 25 (72%) MSSA strains from 15 patients were related to Finnish epidemic MRSA strains, especially to strains FIN-10 and FIN-14. This is in accordance with data from our other study which revealed that despite the wide genomic diversity, many clinical MSSA isolates have genotypes related to nonmultiresistant Finnish epidemic MRSA strains, including the FIN-7 strain (5). It has been shown that major MRSA clones have arisen from a few successful epidemic MSSA clones (3, 13). Nevertheless, in vivo studies have shown that horizontal transfer of SCCmec occurs between staphylococcal species (17), and deletion of the SCCmec region has also been described (1).

Most of the MSSA strains found in this study were genotypically related to the epidemic MRSA strains but only a few of them had received the SCCmec element, and all those strains possessed SCCmec type V. Further studies are needed to explore why some strains get *mecA* and others do not.

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