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Increased incidence of methicillin-resistant *Staphylococcus aureus* ST80, novel ST125 and SCC*mec*IV in the south-eastern part of Norway during a 12-year period

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ABSTRACT

A retrospective population-based study of genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) was performed during the period 1991–2003 in two counties in the south-eastern part of Norway. Isolates of MRSA from all individuals in the two counties in whom MRSA was detected were genotyped by means of multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylococcal protein A gene (*spa*) typing and amplified fragment length polymorphism (AFLP) analysis. Until 1999, only sporadic cases of MRSA infection were reported in these counties, but the incidence increased during the following years. Nine new MLST types were identified in this study. The predominant strains were ST239-MRSA-III, the novel ST125-MRSA-IV, and the central European community-acquired strain ST80-MRSA-IV reported previously. ST80-MRSA-IV was introduced into the two counties in 1997, and the incidence of infections has increased since 2000, so that ST80-MRSA-IV is now the commonest MRSA strain in the region. An increase in MRSA clones carrying SCC*mec*IV has occurred during recent years, which could indicate a shift in the MRSA population in Norway from hospital-acquired MRSA to community-acquired-MRSA.

Keywords Community-acquired MRSA, epidemiology, methicillin-resistant *Staphylococcus aureus*, Norway, SCC*mec* type, sequence types

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) first appeared during the early 1960s [1], and today represents a significant threat to public health in most European countries.

The spread of MRSA in England and Wales during the 1990s is interesting with regard to public health interventions. The proportion of cases of *S. aureus* bacteraemia caused by MRSA increased from 1–2% in 1990–1992 to >40% in 2000, with the emergence of two epidemic MRSA (EMRSA) strains, EMRSA-15 and EMRSA-16, coinciding with this increase [2–6], and the numbers of MRSA infections are still increasing [7,8]. In Norway, the proportion of MRSA involvement in cases of bacteraemia caused by *S. aureus* has remained at *c.* 1% [9]. The reasons for this low percentage could be that Norway has a small population spread over a relatively large area, and that the country is situated geographically on the periphery of Europe. For >10 years, Norway has had MRSA screening programmes for patients admitted to hospitals, and for healthcare workers who have worked in hospitals outside the Nordic countries. To obtain more information concerning the dynamics of the spread of MRSA, it would be interesting to examine the times at which the different epidemic clones of MRSA appeared in Norway, with particular emphasis on the new community-acquired MRSA clones that have been reported on the European continent.

The introduction of molecular typing techniques for epidemiological investigations has provided powerful new tools for tracking the

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origin and routes of dissemination of MRSA. Several methods have been applied to genotype MRSA isolates. However, most such methods depend on comparing DNA fragment patterns, which can lead to difficulties when comparing results from different laboratories. However, multilocus sequence typing (MLST) and staphylococcal protein A gene (*spa*) typing are sequence-based methods that are highly discriminatory and provide universal algorithms that enable easy comparison of the results [10–13]. MLST is based on determining the sequences of internal fragments of seven housekeeping genes, with each fragment being *c.* 450 bp in length [11]. *Spa* typing is based on analysing the polymorphic X region of the staphylococcal protein A gene, situated at the 3′ end of the gene, which consists of a variable number of 24-bp repeats [12].

The aim of the present population-based study was to include isolates from all patients from whom MRSA was isolated during the period 1991–2003. A defined geographical area in Norway with a relatively high population density, excluding the capital Oslo, was chosen for the study. The MRSA isolates from the patients were genotyped and compared with internationally recognised strains using MLST supplemented with staphylococcal cassette chromosome *mec* (SCC*mec*) typing [14–16] and *spa* typing. Amplified fragment length polymorphism (AFLP) analysis [17,18] was also investigated in comparison with MLST and *spa* typing, as AFLP is one of the routine epidemiological typing methods in use at Akershus University Hospital.

MATERIALS AND METHODS

Population, design and bacterial strains

The population of Akershus and Østfold comprises 723 020 inhabitants. These two neighbouring counties are situated in the south-eastern part of Norway, near the Swedish border, and surrounding the capital of Norway, Oslo. The investigation was performed as a retrospective population-based study. All 100 individuals in the region from whom MRSA was isolated between 1991 and 2003 were included, comprising hospitalised patients and patients from the primary healthcare system. All isolates were genotyped (see below). Only one sequence type (ST) was isolated from 99 individuals; the remaining individual yielded two STs (101 isolates in total). The MRSA isolates were obtained from the three microbiological laboratories in the region. All laboratories stored MRSA isolates at − 80°C. The possibility of samples with MRSA being sent to other laboratories in the region was considered to be negligible.

Identification of MRSA

All MRSA isolates were identified following incubation for 2 days on Mueller–Hinton agar containing NaCl 2% w/v, oxacillin 4 mg/L and aztreonam 8 mg/L. Colonies were also tested for oxacillin susceptibility by Etest (AB Biodisk, Solna, Sweden) before storing. Before being genotyped, the MRSA isolates were retested by PCR for the presence of the *mecA* and *nuc* genes [19,20]. PCR was performed in 25-μL volumes containing 20 ng of chromosomal DNA, 5 pmol of each primer, 0.2 mM dNTPs (Invitrogen, Carlsbad, CA, USA), 0.5 U of Platinum *Taq* DNA polymerase (Invitrogen), 2.5 μL of 10× buffer and 3.5 mM $MgCl_2$ (both supplied with Platinum *Taq* DNA polymerase). Amplification was performed in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), with 2 min at 94°C, 30 cycles of 1 min at 94°C, 30 s at 56°C and 1 min at 72°C, and a final extension step of 10 min at 72°C. PCR products were visualised following electrophoresis on agarose 1.5% w/v gels and staining with ethidium bromide 0.5 mg/L using a Gel Doc2000 system (Bio-Rad, Hercules, CA, USA).

Chromosomal DNA isolation

Bacterial cells were resuspended in double-distilled H_2O, to a concentration of *c.* 3×10^8 CFU/mL, and were incubated with lysostaphin (Sigma-Aldrich, St Louis, MO, USA) 0.25 mg/mL at 37°C for 2 h. Chromosomal DNA was then extracted using the GenoM-48 Robotic Workstation and the MagAttract DNA tissue protocol, as recommended by the manufacturer (GenoVision, Oslo, Norway).

MLST was performed according to the protocol of Enright *et al.* [10], with minor modifications in the PCR mastermix. The following seven housekeeping genes were analysed: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyl transferase (*pta*), triosephosphate isomerase (*tpi*) and acetyl coenzyme A acetyltransferase (*yqiL*). PCRs were performed in a final volume of 25 μL containing 25 ng of chromosomal DNA, 0.025 μg of each primer (except for tpi_up and tpi_dn, where 0.25 μg was added), 0.2 mM dNTPs, 0.5 U of Platinum *Taq* DNA polymerase, 2.5 μL of 10× buffer and 1.5 mM $MgCl_2$. The amplified products were purified using a GFX96 PCR Purification Kit (Amersham Biosciences, Chalfont St Giles, UK), followed by sequencing with a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Unincorporated dye terminators were removed from the extension products by isopropanol precipitation, and the sequences of both strands were analysed using an ABI Prism 3100 DNA Genetic Analyzer (Applied Biosystems). The sequencing primers were the same as those used for the initial PCR amplification. The sequences were submitted to the MLST database (http://www.mlst.net), where they were assigned allele and sequence numbers.

SCC*mec* typing

SCC*mec* typing was performed using PCR to detect the presence of the various *ccr* and *mec* complexes. The *ccr* complexes were identified using primers β2, α2, α3 and α4 [14], and the *mec* complexes were identified using primers mI-1, mI-2, mcR2 and mcR3 [15], Is-2, Is-5, mA2 and mA6 [16]. All PCRs were performed in 25-μL reaction volumes containing 10 ng of chromosomal DNA, 5 pmol of each primer, 0.2 mM dNTPs, 0.5 U of Platinum *Taq* DNA polymerase, 2.5 μL of 10×

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