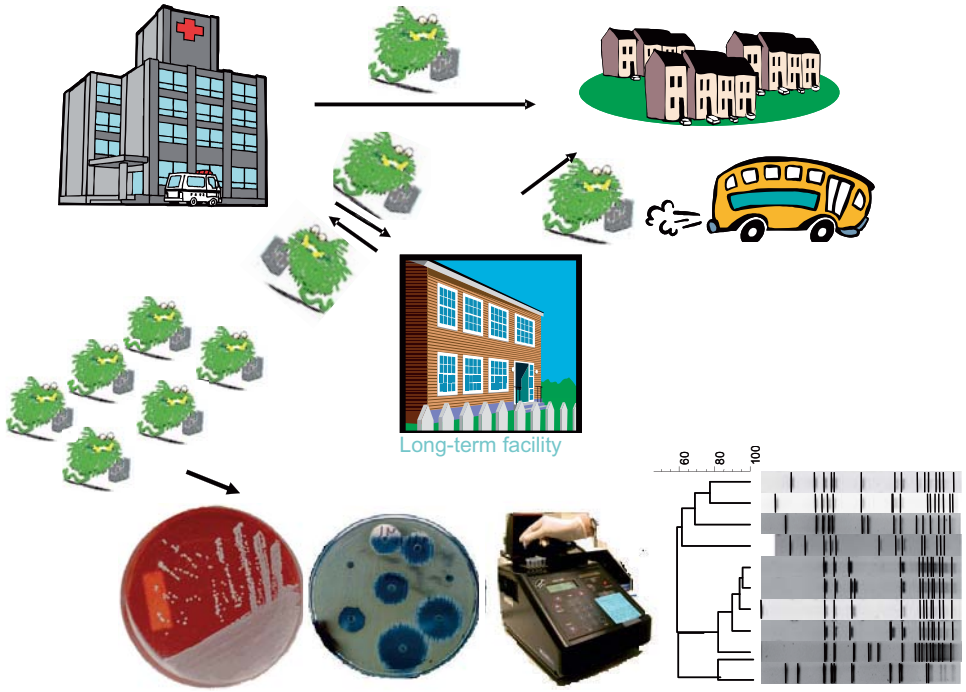

Anne-Marie Kerttula

Methicillin-resistant *Staphylococcus aureus* in Finland: recent changes in the epidemiology, long-term facility aspects, and phenotypic and molecular detection of isolates

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Methicillin-resistant *Staphylococcus aureus* in Finland: recent changes in the epidemiology, long-term facility aspects, and phenotypic and molecular detection of isolates

Anne-Marie Kerttula

ACADEMIC DISSERTATION

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ABSTRACT

Staphylococcus aureus is one of the most important bacteria that cause disease in humans, and methicillin-resistant *S. aureus* (MRSA) has become the most commonly identified antibiotic-resistant pathogen in many parts of the world. MRSA rates have been stable for many years in the Nordic countries and the Netherlands with a low MRSA prevalence in Europe, but in the recent decades, MRSA rates have increased in those low-prevalence countries as well. MRSA has been established as a major hospital pathogen, but has also been found increasingly in long-term facilities (LTF) and in communities of persons with no connections to the health-care setting. In Finland, the annual number of MRSA isolates reported to the National Infectious Disease Register (NIDR) has constantly increased, especially outside the Helsinki metropolitan area. Molecular typing has revealed numerous outbreak strains of MRSA, some of which have previously been associated with community acquisition.

In this work, data on MRSA cases notified to the NIDR and on MRSA strain types identified with pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and staphylococcal cassette chromosome *mec* (SCC*mec*) typing at the National Reference Laboratory (NRL) in Finland from 1997 to 2004 were analyzed. An increasing trend in MRSA incidence in Finland from 1997 to 2004 was shown. In addition, non-multi-drug resistant (NMDR) MRSA isolates, especially those resistant only to methicillin/oxacillin, showed an emerging trend. The predominant MRSA strains changed over time and place, but two internationally spread epidemic strains of MRSA, FIN-16 and FIN-21, were related to the increase detected most recently. Those strains were also one cause of the strikingly increasing invasive MRSA findings. The rise of MRSA strains with SCC*mec* types IV or V, possible community-acquired MRSA was also detected.

With questionnaires, the diagnostic methods used for MRSA identification in Finnish microbiology laboratories and the number of MRSA screening specimens studied were reviewed. Surveys, which focused on the MRSA situation in long-term facilities in 2001 and on the background information of MRSA-positive persons in 2001-2003, were also carried out. The rates of MRSA and screening practices varied widely across geographic regions. Part of the NMDR MRSA strains could remain undetected in some laboratories because of insufficient diagnostic techniques used. The increasing proportion of elderly population carrying MRSA suggests that MRSA is an emerging problem in Finnish long-term facilities. Among the patients, 50% of the specimens were taken on a clinical basis, 43% on a screening basis after exposure to MRSA, 3% on a screening basis because of hospital contact abroad, and 4% for other reasons.

In response to an outbreak of MRSA possessing a new genotype that occurred in a health care ward and in an associated nursing home of a small municipality in Northern Finland in autumn 2003, a point-prevalence survey was performed six months later. In the same study, the molecular epidemiology of MRSA and methicillin-sensitive *S. aureus* (MSSA) strains were also assessed, the results to the national strain collection compared, and the difficulties of MRSA screening with low-level oxacillin-resistant isolates encountered. The original MRSA outbreak in LTF, which consisted of isolates possessing a nationally new PFGE profile (FIN-22) and internationally rare MLST type (ST-27), was confined. Another previously unrecognized MRSA strain was found with additional screening, possibly indicating that current routine MRSA screening methods may be insufficiently

sensitive for strains possessing low-level oxacillin resistance. Most of the MSSA strains found were genotypically related to the epidemic MRSA strains, but only a few of them had received the SCC*mec* element, and all those strains possessed the new SCC*mec* type V.

In the second largest nursing home in Finland, the colonization of *S. aureus* and MRSA, and the role of screening sites along with broth enrichment culture on the sensitivity to detect *S. aureus* were studied. Combining the use of enrichment broth and perineal swabbing, in addition to nostrils and skin lesions swabbing, may be an alternative for throat swabs in the nursing home setting, especially when residents are uncooperative.

Finally, in order to evaluate adequate phenotypic and genotypic methods needed for reliable laboratory diagnostics of MRSA, oxacillin disk diffusion and MIC tests to the cefoxitin disk diffusion method at both +35°C and +30°C, both with or without an addition of sodium chloride (NaCl) to the Müller Hinton test medium, and in-house PCR to two commercial molecular methods (the GenoType® MRSA test and the EVIGENE™ MRSA Detection test) with different bacterial species in addition to *S. aureus* were compared. The cefoxitin disk diffusion method was superior to that of oxacillin disk diffusion and to the MIC tests in predicting *mecA*-mediated resistance in *S. aureus* when incubating at +35°C with or without the addition of NaCl to the test medium. Both the Geno Type® MRSA and EVIGENE™ MRSA Detection tests are usable, accurate, cost-effective, and sufficiently fast methods for rapid MRSA confirmation from a pure culture.

Keywords: methicillin-resistant *Staphylococcus aureus*, MRSA, oxacillin, cefoxitin, epidemiology, long-term facility, molecular method, phenotypic method

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TIIVISTELMÄ

Metisilliini-resistentistä *Staphylococcus aureuksesta* (MRSA) näyttää tulleen pysyvä maailmanlaajuinen ongelma. MRSA on yksi tärkeimmistä tautia aiheuttavista bakteereista ja siitä on tullut yleisin mikrobilääkkeille resistentti patogeeni monissa maissa. Pohjoismaissa ja Hollannissa MRSA-kantoja on pitkään eristetty vähäisiä, tasaisesti nousevia määriä, mutta viime vuosina myös näissä maissa MRSA-kannat ovat selkeästi lisääntyneet. MRSA on ollut jo kauan sairaaloiden vaivana, mutta nykyään sitä löydetään yhä useammin myös pitkäaikaislaitospotilailta sekä henkilöiltä, joilla ei tiedetä olleen yhteyttä terveydenhuollon laitoksiin. Molekyylibiologisten menetelmien avulla on tunnistettu useita MRSA-epidemioita, joista jotkut viittaavat mahdollisiin avohoitoperäisiin tartuntoihin.

Suomessa Kansanterveyslaitoksen (KTL) tartuntatautirekisteriin (ttr) ilmoitettujen MRSA-kantojen lukumäärä on lisääntynyt selkeästi viime vuosina. Samaan aikaan myös kliinisen mikrobiologian laboratorioiden KTL:n referenssilaboratorioon lähettämien MRSA-kantojen määrä on noussut. Tässä väitöskirjassa tutkittiin MRSA:n muuttunutta epidemiologiaa analysoimalla ttr:n ilmoituksia sekä referenssilaboratorioon lähetettyjen kantojen ominaisuuksia Suomessa vuosina 1997-2004. Lisäksi arvioitiin MRSA-diagnostiikan tasoa kliinisen mikrobiologian laboratorioissa, sairaanhoitopiirien MRSA-seulonta-aktiviteettia, ja pitkäaikaishoitolaitosten MRSA-tilannetta vuonna 2001, sekä MRSA-positiivisten henkilöiden näytteenottoperustetta vuosina 2001-2003 kyselytutkimusten avulla.

Eri geenityypitysmenetelmien (PFGE, MLST, SCC*mec*) avulla KTL:n referenssilaboratoriossa todettiin, että vallitsevat MRSA-kannat ovat vaihdelleet vuosittain eri puolilla Suomea, mutta viimeaikaisen nousun takana oli kaksi kansainvälistä moniresistenttiä MRSA-epidemiakantaa. Nämä kaksi kantaa vallitsivat myös jyrkästi lisääntyneissä verilöydöksissä. Myös mahdollisten avohoidon MRSA-kantojen sekä vain beetalaktaameille resistenttien kantojen osuudet lisääntyivät. Kyselytutkimuksen perusteella voitaneen epäillä, että osa näistä vain beetalaktaameille resistenteistä kannoista on saattanut jäädä havaitsematta kliinisen mikrobiologian laboratorioiden riittämättömän MRSA-diagnostiikan vuoksi. Saman kyselyn perusteella voidaan todeta, että MRSA-löydösten määrä on vaihdellut sairaanhoitopiireittäin, mutta se ei ole korreloinut seulonta-aktiviteetin kanssa. Toisen kyselytutkimuksen perusteella voitiin todeta, että MRSA:n lisääntyminen vanhusväestössä viittaa sen aiheuttamaan lisääntyvään ongelmaan vanhainkodeissa. Kolmannesta kyselytutkimuksesta selvisi, että 50 % MRSA-positiivisten potilaiden seulontanäytteistä oli otettu kliinisillä perusteilla, 43 % MRSA-altistumiseen liittyvillä seulontaperusteilla, 3 % ulkomaan sairaalakontakteihin liittyvillä seulontaperusteilla, ja 4 % jonkin muun syyn takia.

Pohjoissuomalaisessa pitkäaikaishoitolaitoksessa syksyllä 2003 tapahtuneen, perimältään omanlaisen ja selvästi rajatun MRSA-epidemian vuoksi haluttiin selvittää tarkemmin pitkäaikaishoitolaitoksen MRSA:n epidemiologiaa, sekä tehdä asukkaista uusi MRSA-seulonta helmikuussa 2004. Lisäksi analysoitiin asukkaiden MRSA- sekä metisilliinille/oksasilliinille herkkien *S. aureusten* (MSSA)-löydösten perimää vertaamalla niitä kansalliseen MRSA-kantakokoelmaan. Uudessa seulonnassa löytyi alkuperäisen MRSA-epidemiakannan lisäksi toinen, Suomesta aiemmin kuvattu MRSA-epidemiakanta. Tämä kanta ei ole mahdollisesti tullut esille laitoksessa tapahtuneissa aikaisemmissa seulonnoissa, koska kannan alhainen resistenssi

metisilliinille/oksasilliinille voi haitata sen löytymistä tavallisella MRSA-seulontamaljalla. Perimäanalyysin perusteella todettiin, että MRSA ja MSSA-kantojen perimä voi olla samanlainen, mutta vain osa MSSA-kannoista on saanut metisilliiniresistenssiä aiheuttavan SCC*mec*-elementin. Tässä tapauksessa kaikilla laitoksesta löytyneillä MRSA-kannoilla oli sama SCC*mec*-tyyppi V kantojen perimästä riippumatta.

Suomen toiseksi suurimmassa vanhainkodissa tutkittiin asukkaiden MSSA- sekä MRSA-kantajuutta. Lisäksi arvioitiin, miten eri näytteenottopaikkojen määrä ja rikastusviljelyn käyttö vaikuttavat *S. aureuksen* (MRSA ja MSSA) löytymiseen. Todettiin, että perineum-näytteiden otto sekä rikasteviljelyn käyttö yhdistettynä nenä- ja haavanäytteiden ottoon voisi olla vaihtoehto nielunäytteiden otolle. Tämä toimisi etenkin asukkailla, joilta näytteenotto ei suju yhteistyökyvyn puutteen vuoksi.

Lopuksi arvioitiin feno- ja genotyyppisiä menetelmiä, jotka olisivat tarkoituksenmukaisia MRSA:n tunnistamisessa kliinisen mikrobiologian laboratorioissa. *S. aureus*-kantojen metisilliini/oksasilliiniherkkyyttä tutkittiin oksasilliini-kiekkotestillä ja -MIC-menetelmällä, sekä kefoksitiini-kiekkotestillä. Menetelmät tehtiin +35°C:ssa ja +30°C:ssa Müller-Hinton agarilla, natriumkloridilisällä tai ilman sitä. Lisäksi verrattiin KTL:n referenssilaboratoriossa käytössä olevaa perinteistä *mecA-nuc*-PCR-menetelmää kaupallisiin GenoType® MRSA- sekä EVIGENE™ MRSA Detection-testeihin. Todettiin, että kefoksitiini-kiekkotesti oli ylivertainen ennustamaan *S. aureuksen* metisilliini/oksasilliiniresistenssiä ja että molemmat kaupalliset genotyyppiset menetelmät olivat käyttökelpoisia, tarkkoja, hinta-laatusuhteeltaan sopivia, sekä riittävän nopeita menetelmiä MRSA:n tunnistamiseen puhdasviljelystä.

Avainsanat: metisilliini-resistentti *Staphylococcus aureus*, MRSA, oksasilliini, kefoksitiini, epidemiologia, pitkäaikaishoitolaitos, tyypitysmenetelmä

CONTENTS

ABBREVIATIONS

agr	accessory gene regulator
aux	auxillary factors for methicillin resistance
BORSA	borderline-resistant *Staphylococcus aureus*
BURST	Based Upon Related Sequence Typing
CA-MRSA	community-acquired methicillin-resistant *Staphylococcus aureus*
CC	clonal complex
CFU	colony forming units
CLED	cystine lactose electrolyte-deficient agar
CLSI	Clinical and Laboratory Standards Institute (formerly NCCLS)
CoNS	coagulase negative *Staphylococcus*
CSF	cerebrospinal fluid
EARSS	European Antibiotic Resistance Surveillance System
fem	factors essential for methicillin resistance
FiRe	Finnish Study Group for Antimicrobial Resistance
HA-MRSA	hospital-acquired MRSA
HCW	health-care ward
HD	hospital district
KTL	National Public Health Institute (Kansanterveyslaitos)
kDa	kilodalton
LTF	long-term facility
Mbp	mega-base-pare
MDR	multi-drug-resistant
*mec*A	gene coding for penicillin-binding protein 2a (PBP2a)
MIC	minimum inhibitory concentration
MH	Müller-Hinton agar
MRSA	methicillin-resistant *Staphylococcus aureus*
GlcNAc	N-acetylglucosamine
MurNAc	N-acetylmuramic acid
NaCl	sodium chloride
NCCLS	National Committee for Clinical Laboratory Standards
NH	nursing home
NIDR	National Infectious Disease Register
NMDR	non-multi-drug-resistant
NNIS	National Nosocomial Infection Surveillance System (USA)
NRL	National Reference Laboratory
nuc	nuclease
MLST	multilocus sequence typing
MSA	mannitol salt agar
ORSAB	Oxacillin Resistance Screening Agar Base
PBP	penicillin-binding protein
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PVL	Panton-Valentine leukocidin
RFLP	restriction fragment length polymorphism
sar	staphylococcal accessory gene regulator
SBA	sheep blood agar
SCC*mec*	staphylococcal cassette chromosome *mec*
SRE	right extremity regions

ST	sequence type
TSST-1	toxic shock syndrome toxin-1
VISA	vancomycin intermediate-resistant *Staphylococcus aureus*
VRSA	vancomycin-resistant *Staphylococcus aureus*

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text with Roman numerals I-V.

I Kerttula A-M., Lyytikäinen O., Salmenlinna S., Vuopio-Varkila J.: Changing epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Finland. J Hosp Inf 2004. 58(2): 109-114

II Kerttula A-M., Lyytikäinen O., Kardén-Lilja M., Ibrahem S., Salmenlinna S., Virolainen A., Vuopio-Varkila J.: Nationwide Trends in Molecular Epidemiology of Methicillin-resistant *Staphylococcus aureus*, Finland, 1997-2004 (in press: BMC Inf Dis 2007, 7:94).

III Kerttula A-M., Lyytikäinen O., Vuopio-Varkila J., Ibrahem S., Agthe N., Broas M., Jägerroos H., Virolainen A.: Molecular epidemiology of an outbreak caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in a health care ward and associated nursing home. J Clin Microbiol 2005. 43(12): 6161-63.

IV Kerttula A-M., Lyytikäinen O., Virolainen A, Finne-Soveri H., Agthe N., Vuopio-Varkila J.: *Staphylococcus aureus* colonization among nursing home residents in a large Finnish nursing home (in press: Scand J Infect Dis).

V Kerttula A-M., Vainio A., Mero S., Pasanen T., Vuopio-Varkila J., Virolainen A.: Evaluation of molecular and phenotypic methods for screening and detection of methicillin-resistant *Staphylococcus aureus* (submitted).

 In addition, this thesis also presents some unpublished results.

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most commonly identified antibiotic-resistant pathogens in many parts of the world, including Europe, the Americas, North Africa, the Middle East, and East Asia. In recent decades, MRSA rates have been increasing worldwide, including in the Nordic countries and the Netherlands, where MRSA rates have been low and stable for many years (Fridkin et al., 2002; Tiemersma et al., 2004; Turnidge and Bell, 2000). Evidence suggests that MRSA infections of nosocomial origin increase morbidity, mortality, and costs (Cosgrove et al., 2005; Cosgrove et al., 2003; Engemann et al., 2003), and may cause patient suffering and harm both psychologically and financially (Tarzi et al., 2001).

Infections caused by MRSA can also be acquired outside health care settings, and specifically in the community. Community-acquired (CA) MRSA has most often been reported in persons or groups with a high intensity of physical contact, which may aid transmission (CDC, 2003a; CDC, 2003b; Groom et al., 2001; Zinderman et al., 2004). When hospital-acquired (HA) MRSA strains are known to spread to the community, community-based strains may also migrate into health care settings, thus creating a two-way flow of MRSA (de Trindade et al., 2005; Kilic et al., 2006; Seybold et al., 2006). Some researchers have suggested that long-term facilities (LTF) such as nursing homes (NH) may be reservoirs of MRSA and, therefore, be responsible for the introduction of this organism into the health care setting (Bradley, 1999). Controlling infection in NHs is challenging because residents should be able to live as normal a life as possible.

Active surveillance cultures for patients at high risk for MRSA colonization or infection, together with contact precautions, are essential to decrease the incidence of nosocomial MRSA infections (Muto et al., 2003). Clinical microbiology laboratories play a pivotal role in the diagnosis and antibiotic susceptibility testing of MRSA. Low-level oxacillin resistance among MRSA is a particular diagnostics problem, and in practice this problem becomes even more important when *S. aureus* strains are resistant only to oxacillin (Fang and Hedin, 2003; Merlino et al., 2002b; Safdar et al., 2003). False negative results may lead to treatment failure and the spread of MRSA due to miscalculation of infection control practices. On the other hand, MRSA strains susceptible to very few antibiotics exist. Vancomycin, a glycopeptide antibiotic, is the treatment of choice for serious MRSA infections, especially those with multi-drug-resistant strains. In 1996, however, the first clinical vancomycin intermediate-resistant *S. aureus* (VISA) was documented (Hiramatsu et al., 1997), and VISA strains have subsequently been isolated around the world, although they have remained rare (Walsh and Howe, 2002). In addition, three *S. aureus* strains with full resistance to vancomycin (VRSA) have been reported thus far (CDC, 2002a; CDC, 2002b; CDC, 2004; Chang et al., 2003; Tenover et al., 2004).

A functional, active surveillance system is needed for monitoring the constantly changing epidemiology of MRSA (Coia et al., 2006; Gould, 2005). Outbreak investigation and consecutive control measures have been proven to be cost-effective, at least in situations with a low-prevalence of MRSA (Björholt and Haglind, 2004; Vriens et al., 2002). The ability to discriminate accurately between MRSA isolates is crucial in investigating their spread on a local level, and pulsed-field gel electrophoresis (PFGE) has been lauded as the gold standard for that purpose (Tenover et al., 1994). When looking at the global epidemiology of MRSA (Robinson and Enright, 2003), multi-locus sequence typing (MLST) and the mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*) -typing (Enright et al., 2000; Ito et al., 2004; Oliveira and de Lencastre, 2002) are more useful.

Because continuously increasing numbers of MRSA cases were reported to the National Infectious Disease Register (NIDR) at the National Public Health Institute (KTL), and an equal number of MRSA isolates were sent to the National Reference Laboratory (NRL) for further confirmation and typing, the reasons behind the changing epidemiology of MRSA were investigated. The data obtained from the NIDR and NRL from 1997 to 2004 was analyzed, the studies of *S. aureus*/MRSA colonization and prevalence in nursing homes using molecular typing techniques were performed and the screening methods were assessed, and the phenotypic and genotypic methods of detecting MRSA were evaluated, in an attempt to bring appropriate information to clinical microbiology laboratories.

2. REVIEW OF THE LITERATURE

2.1 *Staphylococcus aureus*

2.1.1 Structure, virulence factors, and pathogenesis

Staphylococcus aureus is a facultative aerobic gram-positive coccus living alongside humans and animals as an opportunistic pathogen. *S. aureus* consists of cytosol, a single cytoplasmic membrane, and the surrounding cell wall. The cell wall of *S. aureus* is mainly peptidoglycan, composed of repeating disaccharide N-acetylglucosamine-N-acetylmuramic acid (GlcNAc-MurNAc) units with attached teichoic acids (Navarre and Schneewind, 1999). Glycan chains are crosslinked by tetrapeptides consisting of L-alanine, D-glutamate, L-lycine, and L-alanine to pentaglycine interbridge, linked to wall peptide. The main function of peptidoglycan is to provide a rigid envelope for the cell contest. Peptidoglycan also has endotoxic properties, and has been reported to cause organ dysfunctions in experimental animals (Holtfreter and Broker, 2005). There are numerous other bacterial components and secreted products, such as virulence factors, that affect the pathogenesis of *S. aureus*. Teichoic acids, another cell wall component, have been found not only to establish nasal colonization (Aly and Levit, 1987; Weidenmaier et al., 2004), but also to contribute together with peptidoglycan to the severity of staphylococcal sepsis (De Kimpe et al., 1995). The cell wall assembly is catalysed by high molecular weight bifunctional enzymes, penicillin-binding proteins (PBP). Four native forms of PBPs (1-4) promote the polymerization of glycan from its disaccharide precursor, and from the transpeptidation of wall peptides (Navarre and Schneewind, 1999). Most *S. aureus* strains produce a slimy, extracellular capsular polysaccharide. A total of eight capsular serotypes have been described, and serotypes 5 and 8 account for approximately 25% and 50%, respectively, of isolates found in humans (O'Riordan and Lee, 2004).

The adherence of *S. aureus* to host tissue is an important step in pathogenesis as well as in colonization. Surface proteins such as protein A, clumping factors, fibronectin-binding proteins, and collagen-binding proteins can adhere to extracellular matrix components of the host (Foster and Hook, 1998). The main function of protein A, however, is to bind the IgG Fc-domaine. Almost all strains produce and secrete enzymes and exotoxins including hemolysins (alpha, beta, gamma, and delta), proteases, lipases, nucleases, hyalonuridase, and collagenase (Dinges et al., 2000). Some toxins, such as toxic shock syndrome toxins (TSST-1) or enterotoxins (SEA, SEB, SECn, SED, SEE, SEG, SHE, and SEI) produced by some strains, are sufficient to cause specific diseases, whereas no single virulence factor has been shown to cause an inflammatory process. For example, to cause systemic diseases such as bacteremia, *S. aureus* must produce components capable of attaching to cells or tissues, factors that decrease phagocytosis in order to escape the host immune system, and to modify proteases, exotoxins, and enzymes, causing tissue damage, and thus, allowing the dissemination of *S. aureus*. Some strains also produce exfoliative toxins (ETA and ETB), and leukocidins such as Panton-Valentine leukocidin (PVL). PVL genes encode a bicomponent leukotoxin, which comprises pore-forming staphylococcal toxins together with alpha- and gamma-haemolysins (Prevost et al., 2001). To date, 11 leukotoxin proteins have been identified: six of class S proteins (LukSPV, LukE, LukM, HlgA, HlgC, and LukSI) and five of class F proteins (LukFPV, LukD, LukF'PV, HlgB, and LukFI) (Prevost et al., 2001). These proteins may be required to break the tissue down into nutrient components for bacterial growth. The primary function of these toxins may still be to inhibit the host immune response, although they also have potent effects on cells. Injecting PVL into the skin of rabbits has been reported to cause dermal necrosis (Ward and Turner, 1980), suggesting that it may cause severe skin infections in humans.

Studies have reported an association between PVL containing MRSA strains and virulent necrotizing pneumonia (Gillet et al., 2002).

2.1.2 Genome

The genome of *S. aureus* consists of a single circular chromosome (2.7-2.9 Mbp) containing about 2600 genes. Whole genome sequences have been reported for nine *S. aureus* isolates: COL, NCTC 8325, N325, Mu50, MW2, MRSA252, MSSA476, RF122, and USA300-FPR3757 (http://cmr.tigr.org/tigr-scripts/CMR/shared/Genomes.cgi/, http://www.genome.ou.edu/staph.html/, http://www.sanger.ac.uk/Projects/S_aureus/). Phylogenetic studies have indicated that more than half of the predicted proteins encoded by the *S. aureus* genome are similar to those of *Bacillus subtilis* and *Bacillus halodurans* (Kuroda et al., 2001). These typically include proteins encoded by house-keeping genes essential for absorbing nutrients from the environment, metabolic intermediate synthesis, and reproduction. Eight genomic islands conferring pathogenity or antibiotic resistance have been found on the *S. aureus* chromosome (Ito et al., 2003). There is also an assortment of extrachromosomal accessory genetic elements: conjugative and nonconjugative plasmids, mobile elements, prophages and other variable elements.

2.1.3 Carriage

Humans are a natural reservoir of *S. aureus* and the primary ecological niches of *S. aureus* are the anterior nares, although other body sites such as the throat, perineum, groin, and skin may also be colonized with *S. aureus*. *S. aureus* nasal carriage has been identified as a major risk factor in the development of infections not only in the hospital setting (Corbella et al., 1997; Kluytmans et al., 1995; von Eiff et al., 2001), but in the community as well (Wertheim et al., 2005). Many underlying diseases or conditions such as insulin-dependent diabetes mellitus, continuous ambulatory peritoneal dialysis (CAPD), intravenous drug abuse, human immunodeficiency virus (HIV) infection or AIDS, and *S. aureus* skin infections and other skin diseases have been associated with a higher *S. aureus* nasal carriage and subsequent infection rate (Berman et al., 1987; Luzar et al., 1990; Nguyen et al., 1999; Williams et al., 1998). According to cross-sectional studies, a mean carriage rate of 37% was found when investigating the prevalence and incidence of *S. aureus* nasal carriage (Kluytmans et al., 1997). However, the range has been reported to be large: 9-100%. The reason for such a high range of carriage rates may be due to differences in the quality of sampling and in the laboratory techniques used to culture specimens. The *S. aureus* nasal carriage rate may also have changed over the years; previous studies have reported higher rates than those published recently (Kluytmans et al., 1997; Wertheim et al., 2005).

Nasal carriage patterns differ between healthy persons, and persistent carriage has been reported in 10-35% of individuals, 20-75% carry *S aureus* intermittently, and 5-50% never carry *S. aureus* (Kluytmans et al., 1997). The non-carrier state may be attributable to bacterial interference with each other: when the ecological niche is already occupied by other bacteria such as coagulase-negative staphylococci (CoNS) or *Corynebacterium* species, *S. aureus* does not seem to replace the resident bacterial population (Hu et al., 1995). Persistent carriage is more common in young children than in adults, and the carriage pattern has been reported to change in many persons between age 10-20 (Armstrong-Esther, 1976). In addition, a persistent carriage rate is higher in males than in females, and depends on hormonal status (Eriksen et al., 1995; Winkler et al., 1990). Significantly higher numbers of *S. aureus* bacteria have been reported in the nostrils of persistent carriers than in those of intermittent carriers, which results in an increased risk of *S. aureus*

infections; elderly healthy persistent carriers had higher amounts of *S. aureus* bacteria than did young carriers (Nouwen et al., 2004). Based on molecular studies, the exchange rate of *S. aureus* strains has been reported to be significantly higher in intermittent carriers than in persistent carriers (van Belkum et al., 1997; VandenBergh et al., 1999). No common genetic or phenotypic characteristics segregating persistent from intermittent colonizing strains have been found thus far, although much research has focused on specific staphylococcal factors and interactions with host components (Biesbrock et al., 1991; Patti et al., 1994; Sanford et al., 1989; van Belkum et al., 1997).

Cespedes and colleagues have developed a mathematical model for investigating the frequency of the simultaneous nasal carriage of multiple strains of *S. aureus* (Cespedes et al., 2005). According to that study, 6.6% of *S. aureus*-colonized individuals carry more than one strain. The presence of more than one strain of *S. aureus* at the same time increases the potential for the horizontal transfer of genes, including virulence determinants or antimicrobial-resistance genes. This may be a problem when a single antibiotic-susceptible isolate, rather than another, more resistant strain from patients infected with *S. aureus* is only detected. The treatment may therefore be unsuitable.

2.1.4 *S. aureus* infections

S. aureus is one of the most important bacteria that cause disease in humans. It is the most common cause of skin and soft tissue infections such as abscesses, carbuncles, folliculitis, furuncles, impetigo, bullous impetigo, and cellulitis. Most skin infections resolve without treatment within a few weeks, although some infections require incision and drainage or antibiotics to cure the infection. Skin infections left untreated can, however, develop into more serious infections such as bloodstream infection and septic shock (Lina et al., 1999; Lindenmayer et al., 1998). Other life-threatening infections caused by *S. aureus* include endocarditis, pneumonia, scalded skin syndrome, or bone and joint infections. Serious infections typically require hospitalization and treatment with intravenous antibiotics. *S. aureus* was the most frequently isolated pathogen from skin and soft tissue infections in the USA, Canada, Latin America, Western Pacific and Europe, and was the most commonly found pathogen causing bloodstream infection and pneumonia in almost all geographic areas (Diekema et al., 2001). In Finland, *S. aureus* has been reported to cause 700-900 septic infections annually (Lyytikäinen et al., 2002), and to be the second most common pathogen causing hospital-acquired infections in general (Lyytikäinen, 2005). In addition, the annual incidence of bloodstream infections caused by *S. aureus* has risen from 11 per 100,000 population in 1995 to 17 in 2001, and the increase was most distinct in patients over 74 years of age (Lyytikäinen et al., 2005). Catheter-related infections and postoperative wound infections are also commonly associated with *S. aureus*. In fact, intravascular catheter-related infections are the primary cause of nosocomial bacteremia (Eggimann and Pittet, 2002). Toxic shock syndrome (TSS) and food poisoning are toxin-mediated diseases caused by TSST-1 and enterotoxin-producing strains of *S. aureus*, respectively. TSS is an acute, multisystem disease with symptoms such as high fever, hypotension, desquamation of the skin, and dysfunction of multiple organ systems (Dinges et al., 2000). Although TSS is most commonly believed to be related to tampon use, toxin-producing *S. aureus* strains have also been isolated from children with major systemic non-invasive illness (Dinges et al., 2000). *S. aureus* can grow in a variety of foods because it withstands a wide range of temperatures, pH conditions, and sodium chloride (NaCl) concentrations (Le Loir et al., 2003).The symptoms of food poisoning caused by *S. aureus* include abdominal cramps, nausea, and vomiting, sometimes followed by diarrhea. The onset of symptoms is rapid (from 30 min to 8 h) and the symptoms will usually pass spontaneously after 24 h.

2.2 Methicillin-resistant *S. aureus*

2.2.1 Methicillin resistance

Methicillin resistance in staphylococci is due primarily to the acquisition of a mobile staphylococcal chromosomal cassette which carries the *mec*A-gene, known as SCC*mec* (Katayama et al., 2000). This *mec*A-gene encodes an altered PBP, PBP2a (or PBP2'), that is 78 kDa in size (Hartman and Tomasz, 1984). Methicillin, like all beta-lactam antibiotics, mimics the structure of D-alanine-D-alanyl, which is the target of a transpeptidation reaction catalyzed by PBPs (Navarre and Schneewind, 1999). The affinity of beta-lactams towards PBP2a is much lower than towards native PBP2, thus allowing continuous cell wall assembly (Reynolds and Brown, 1985).

To date, researchers have identified five types of SCC*mec* elements (Figure 1) and several variants (Boyle-Vavra et al., 2005; Ito et al., 2001; Ito et al., 2004; Ma et al., 2002; Oliveira and de Lencastre, 2002; Oliveira et al., 2001; Shore et al., 2005). Each SCC*mec* type integrates at the same site (*attBscc*) located near the *S. aureus* origin of replication in the *orfX* gene of unknown function (Ito et al., 1999). For movement, SCC*mec* carries three specific genes, designated cassette chromosome recombinases A, B, and C2 (*ccrA*, *ccrB*, and *ccrC*), which encode recombinases of the invertase/resolvase family (Boyle-Vavra et al., 2005; Ito et al., 1999; Ito et al., 2004). In addition to the *mec*A gene, the class A *mec* gene complex contains two intact genes; *mecI* encodes a transcription repressor protein, and *mecR1*, a signal-transduction protein (Hiramatsu et al., 2001). Class B *mec* complex contains *mecA*, but *mecI* and part of the *mecR1* are deleted, and a truncated copy of an insertion sequence, IS1272, is integrated into this deletion. Class C2 *mec* comprises of a copy of insertion sequence IS*431*, *mecA*, a truncated copy of *mecR1*, and another copy of IS*431* (SCC*mec* V) (Ito et al., 2004) or of truncated transposase in IS*431* (SCC*mec* V_T) (Boyle-Vavra et al., 2005).

Type I SCC*mec* carries class B *mec* and type 1 ccr; type II SCC*mec* possesses class A *mec* and type 2 *ccr*; type III SCC*mec* has class A *mec* and type 3 *ccr*; type IV contains class B *mec* and type 2 *ccr*; and type V comprises class C *mec* and type 5 *ccr*. The *mec*A gene is the only resistance gene involved in types I, IV, and V SCC*mec* elements. SCC*mec* types II, III, and IIIA, on the contrary, carry phage Tn554, which encodes for resistance to macrolides, lincosamines and streptogramins. In addition, both SCC*mec* IA and II carry phages pUB110, which encodes for resistance to tobramycin, and pG01, which encodes resistance to gentamycin and trimetoprim. SCC*mec* III carries pT181 encoding resistance for tetracycline (Deresinski, 2005; Hiramatsu et al., 2001). In general, SCC*mec* types I, II and III are considered mainly as hospital-acquired (HA), and SCC*mec* types IV and V as community-acquired (CA).

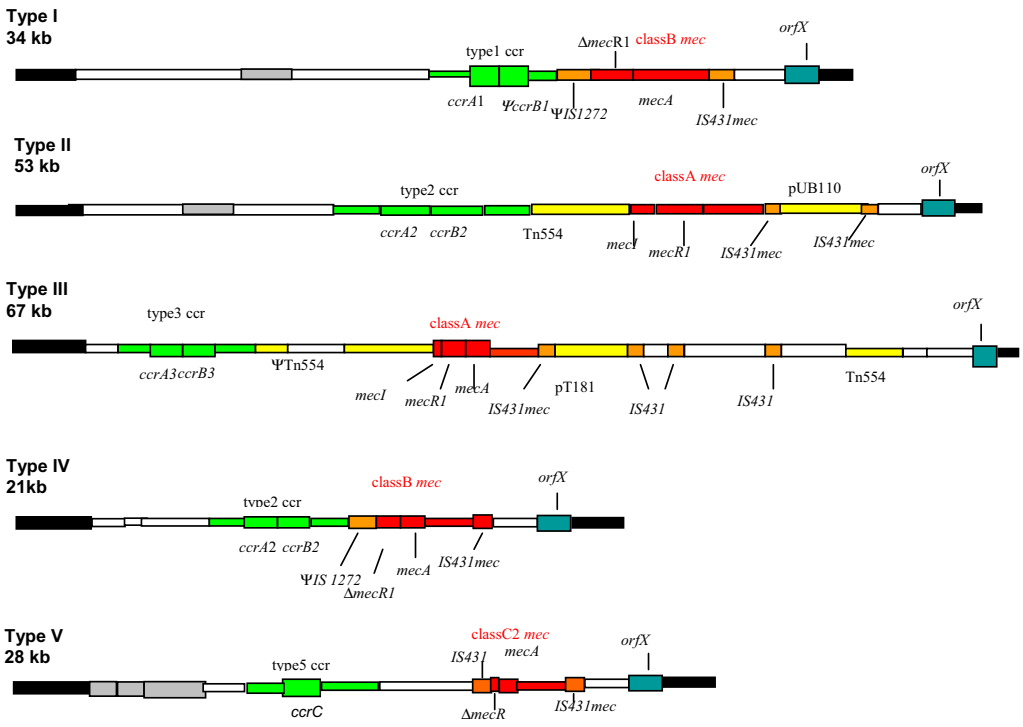

Figure 1. SCC*mec* types I-V according to published strain types. Type I, strain NCTC10442 (Ito, 2004); type II strain N315 (Ito, 1999); type III strain 85/2082 (Ito, 2001); type IV, strain 8/6-3P (Ma, 2002); type V, strain WIS (Ito, 2004). The *mec* gene complex appears in red, the *ccr* gene complex in green, insertion sequences (*IS431* or *IS1272*) in orange, elements encoding resistance to antimicrobials other than betalactams (Tn554, ΨTn554, pUB110, and pT181) in yellow, the *orfX* gene of unknown function in blue, and other elements (genes) in white or in grey.

Various SCC*mec* types are widely distributed among staphylococcal species other than *S. aureus*. Several findings support the understanding that the intra- and interspecies transfer of genetic information may occur through a mobile SSC element. First, the origin of SCC*mec* is unknown, but a close homologue of the *S. aureus mec*A gene has been identified in *S. sciuri*, of both animal and human origin (Couto et al., 1996; Couto et al., 2000). Second, since the IS1272 element seems to be intact and exists in multiple copies in the genome of *S. haemolyticus*, IS1272 usually contains deletions in *S. aureus* and in *Staphylococcus epidermidis*. Thus, some researchers have suggested that *S. haemolyticus* acquired IS1272 before *S. aureus* and *Staphylococcus epidermidis* did (Archer et al., 1996; Kobayashi et al., 1999). Third, one recent study has reported an MRSA *in-vivo* formation by the horizontal transfer of *mec*A during antibiotic treatment between *Staphylococcus epidermidis* and *S. aureus* (Wielders et al., 2001). Fourth, an SCC without the *mec*A gene complex has been identified in *S. aureus* strain, which carries a *cap* operon instead of an antibiotic resistance gene (Luong et al., 2002). Finally, clinically significant *Staphylococcus epidermidis* isolates were reported to harbour SCC*mec* type IV, which was identical in size and 98% homologous to DNA sequences for *S. aureus* (Wisplinghoff et al., 2003).

Many clinical MRSA isolates express resistance to methicillin heterogeneously. This means that the majority of cells are susceptible to low concentrations of methicillin, and only a minority of cells can grow at high concentrations. In addition to *mec*A, other resistance mechanisms are also needed

for the expression of methicillin resistance in *S. aureus*. Factors essential to methicillin resistance (*fem*) or auxillary (*aux*) factors involved in peptidoglycan synthesis include normal chromosomal genes of *S. aureus*, of which defective mutants inhibit precursor formation. In addition, *ilm* and global regulators *agr* and *sar* genes may influence methicillin resistance (Berger-Bachi, 1994; Chambers, 1997). Deletions and mutations in *mecI* or mutations in the promoter region of *mecA* may result in heterogeneous resistance (Kobayashi et al., 1998; Shukla et al., 2004; Suzuki et al., 1993). In addition, partial deletion of the regulatory genes and absence of beta-lactamase regulatory genes (*blaI* and *blaR1*) result in the constitutive production of PBP2a (Hackbarth and Chambers, 1993). The production of PBP2a, however, is strongly repressed in MRSA strains containing fully functional and intact *mec* regulatory genes. Those isolates, although possessing *mecA*, seem to be susceptible by conventional testing. Confusion sometimes arises in the terminology of heterogeneous resistance and borderline resistance. Borderline resistance in *S. aureus* isolates is due to either penicillinase hyper-production (termed BORSA) or to modifications of the PBPs (sometimes termed MODSA). Borderline-resistant *S. aureus* strains never carry *mecA* gene, and are therefore not MRSA.

2.2.2 Public health importance

Methicillin was first introduced in 1959 to treat infections caused by penicillin-resistant *S. aureus*. In 1961, reports emerged from the UK of *S. aureus* isolates resistant to that new antibiotic (Jevons, 1961). Soon MRSA strains were found in other European countries, and later in Japan, Australia and the USA. Currently, MRSA is the most commonly identified antibiotic-resistant pathogen in US hospitals (Diekema et al., 2004a; NNIS, 2004), and its proportion in intensive care units has increased from 35.9% in 1992 to 64.4% in 2003 (Klevens et al., 2006). In Europe, the proportion of invasive isolates resistant to oxacillin, another anti-staphylococcal penicillin which later became a substitute for methicillin, varies from <1% to >50% (EARSS, 2006). In the UK, for example, the proportion of invasive MRSA isolates has increased from 2% in 1990 to a peak of 43% in 2002, with a slight decline thereafter (Johnson et al., 2005), whereas in the Nordic countries and the Netherlands, the proportion remains less than 5% (EARSS, 2006). Reports indicate that mortality due to MRSA infections increased in England and Wales during the period 1993-2005 (Crowcroft and Catchpole, 2002; NationalStatistics, 2007). A meta-analysis study estimated the death rate for patients with MRSA bacteremia to be approximately twice higher than the death rate due to the bacteremia caused by methicillin-sensitive *S. aureus* (MSSA) (Cosgrove et al., 2003), although no greater intrinsic virulence of MRSA has been detected (Hershow et al., 1992). In addition, MRSA infections are responsible for lengthier periods of hospitalization and increased costs (Cosgrove et al., 2005). Reports attribute extra costs to extra days spent in hospital, antimicrobials for treating the infection, and laboratory diagnostics (Björholt and Haglind, 2004; Muto et al., 2003).

In general, countries with stringent control measures tend to report low MRSA incidence rates (Salmenlinna et al., 2000; Verhoef et al., 1999). The 'search and destroy' policy applied in the Nordic countries and the Netherlands requires that all patients at risk for MRSA carriage be isolated and screened before admission to hospital (EARSS, 2006). To recognize outbreaks, to reduce infection rates and thereby morbidity, to improve care, and to reduce costs in health care settings requires a functional surveillance system (Roy and Perl, 1997). Systematic data collection from participating instances in the surveillance system, epidemiological and microbial data analysis, and information feedback on the instances providing the data are the key steps of an effective surveillance system. The precise definitions and methods used, however, depend on the surveillance demanded. Surveillance can be performed at the local, national or international level, and may be restricted to certain units or other restricted areas. For example, National Nosocomial Infection

Systems (NNIS) collects and analyses data from intensive care units, high-risk nurseries, and surgical patients in US hospitals (NNIS, 2004). In addition, the international antimicrobial surveillance system European Antibiotic Resistance Surveillance System (EARSS) performs continuous surveillance of the seven most important bacterial pathogens that cause invasive infections and monitors variations in antimicrobial resistance over time and place (www.earss.rivm.nl). Moreover, The SENTRY Antimicrobial Surveillance Program collects antimicrobial resistance data on bacteria responsible for bloodstream infections, skin and soft-tissue infections, and pneumonia (Diekema et al., 2000; Diekema et al., 2001; Pfaller et al., 1998; Pfaller et al., 1999).

Besides being a significant problem in hospitals, MRSA can cause infections in the community. Such novel MRSA strains were observed for the first time in 1993 among Indigenous Australian patients who had had no previous contact with health care systems (Udo et al., 1993). In 1999, four pediatric deaths resulting from CA-MRSA infections were reported, thus generating greatly increased interest in this organism among health care providers (CDC, 1999). Recently, increasing numbers of MRSA infections from persons unassociated with health care facilities have been reported worldwide (Aires de Sousa et al., 2005; Borer et al., 2002; Bratu et al., 2005; Carleton et al., 2004; Harbarth et al., 2005; Hsu et al., 2006; Ma et al., 2005; Mishaan et al., 2005; Mulvey et al., 2005; O'Brien et al., 2004; Salmenlinna et al., 2002; Söderquist et al., 2006; Takizawa et al., 2005; Urth et al., 2005). Outbreaks of CA-MRSA infections, especially skin and soft-tissue infections, have been described among members living in closed communities or having had direct skin-to-skin contact, as with competing athletes, prison inmates, military recruits, and children in child care centers (CDC, 2003a; CDC, 2003b; Lindenmayer et al., 1998; Shahin et al., 1999; Zinderman et al., 2004).

The SCC*mec* types IV and V typical of CA-MRSA are quite small and can easily be packed and transferred horizontally to other staphylococcal strains. The proportional increase of MRSA strains susceptible to a wide variety of antimicrobials, which are probable CA-MRSA isolates, suggests that the epidemiology of MRSA is changing. One study suggested that CA-MRSA strains may be better able to adhere to epithelial cells than are HA-acquired MRSA strains (Adhikari et al., 2002). Reports of the CA-MRSA strains' higher tolerance for salt than that of nosocomial strains suggest that it may improve its ability to survive as skin flora (Adhikari et al., 2002). The ability of CA-MRSA strains to colonize hosts in the community and to cause infections is mediated by several virulence factors. Analysis of the genome of one CA-MRSA strain, MW2, demonstrates 19 unique genes encoding virulence factors not found on other *S. aureus* genomes sequenced thus far (Baba et al., 2002). The most well-known CA-MRSA virulence factor is PVL, which is a potent necrotizing toxin. Furunculosis is the most frequently reported presentation of CA-MRSA infection, and most of the CA-MRSA strains that cause epidemic furunculosis carry PVL genes (Lina et al., 1999; Zetola et al., 2005). The definitions of CA-MRSA used in the literature have varied over the years (Salgado et al., 2003; Zetola et al., 2005). Epidemiologically, all MRSA infections acquired outside of the health care setting or acquired within 48-72 h after hospitalization are by definition community-acquired. This may, however, overestimate the problem of CA-MRSA if data from previous health care contacts are unavailable (Salgado et al., 2003). The presence of SCC*mec* types IV or V, and/or the expression of *lukS-PV* and *lukF-PV* genes that encode for PVL, along with epidemiological data is also used in defining of CA-MRSA (Coombs et al., 2004; Diep et al., 2004; Liassine et al., 2004; Vandenesch et al., 2003). Although many strains found in the community harbor these markers, the transmission of "CA-MRSA" strains between health care settings and the community may occur (de Trindade et al., 2005; Kilic et al., 2006; Seybold et al., 2006).

2.3 MRSA in long-term facilities

S. aureus infection is a serious problem among the elderly in acute care. Complications and death from the most severe staphylococcal infections such as endocarditis, pneumonia, meningitis, and septic arthritis are common among persons over the age of 65 (Espersen et al., 1991; Jensen et al., 1993; McGuire and Kauffman, 1985; Terpenning et al., 1987; Terpenning et al., 1988). With the increasing number of elderly people whose care has been moved outside of acute care, *S. aureus* and especially MRSA infections have become a major issue in LTF.

2.3.1 Epidemiology

Colonization with *S. aureus* occurs predominantly in the nostrils, skin, rectum, and perineum (Bradley, 1999). MRSA colonization rates in LTF vary, but the highest colonization rates have been obtained from samples taken from the nostrils and wounds. Previous studies from the USA, the UK, Australia, and Japan have reported the prevalence of MRSA among residents to vary from 8% to 53% (Bradley, 1997), whereas recently published, mainly European studies have reported a prevalence of 0.7% to 10.1% (Cretnik et al., 2005; de Neeling, 2003; Hoefnagels-Schuermans et al., 2002; Mendelson et al., 2003; O'Sullivan and Keane, 2000b; von Baum et al., 2002). Differences in colonization rates may depend on a variety of factors such as the prevalence of MRSA in transferring from one health care institution to another, the presence of an outbreak of MRSA infections, the type and severity of the residents' underlying condition, and the infection control practices at the LTF (Bradley, 1999). In a nursing home (NH) where MRSA was endemic and multiple sites of residents were cultured monthly, 65% of patients never acquired MRSA, 25% were colonized with MRSA before admission to the nursing home, and 10% acquired MRSA during their nursing home stay (Bradley et al., 1991). Longitudinal studies indicate that, once colonized among NH residents, colonization seems to be persistent (Bradley et al., 1991; Mulhausen et al., 1996). In endemic settings, residents have been reported to be colonized with many different MRSA strains (Bradley et al., 1991; Drinka et al., 2005; Muder et al., 1991) in contrast to epidemic situations where one or two strains circulate. MRSA colonization is probably common in LTF because, along with the potential for acquisition in the NH itself, residents already colonized from other facilities continue to be admitted, MRSA carriage persists, and the length of stays are long.

2.3.2 Risk factors

Not all residents in NHs appear to be at the same risk for colonization by MRSA. Several studies have identified current antibiotic therapy, male gender, the presence of invasive devices, the presence of pressure sores or wounds, the presence of catheters, hospital admission, and prior MRSA colonization as risk factors (Hsu, 1991; Mendelson et al., 2003; Niclaes et al., 1999; O'Sullivan and Keane, 2000a; von Baum et al., 2002; Vovko et al., 2005). Persons colonized with *S. aureus* or MRSA are generally at increased risk of becoming infected. In such cases, the infection is typically caused by the colonizing strain. Rates of MRSA infections, however, do not seem to approach those of asymptomatic MRSA colonization (Bradley, 1997; Bradley, 1999; Bradley et al., 1991; Muder et al., 1991). More than half of these infections involve skin and soft tissue or urinary track infections, and hospital care is required primarily for the administration of intravenous antimicrobials. Although MRSA infection rates are low in NHs, studies show that residents colonized with MRSA have a four- to six-fold higher risk of developing an MRSA infection than do noncolonized residents (Muder et al., 1991; Mulhausen et al., 1996).

2.3.3 Management

Interest in the epidemiology of infection and its control in LTFs and NHs has grown in recent decades as increasing numbers of elderly people are treated in acute and high-risk units, and then transferred to LTFs. In addition to hospital-acquired MRSA, LTFs with endemic MRSA may represent a reservoir of MRSA, and therefore create a two-way flow of MRSA between hospitals and LTFs. To prevent the transmission of MRSA between patient and health care staff, appropriate infection control practises must exist (Bradley, 1999). Lack of resources may be one of the major limitations in infection control practices; a full-time infection control practitioner with formal training and optimal patient-to-nurse ratios are only seldom available. Thus, infection control procedures must be sufficiently simple for health care technicians and other non-medically educated personnel to follow. In general, functional control precautions of acute care may be adapted to LTF (MRSA-asiantuntijatyöryhmä, 2004; Muto et al., 2003). There are, however, some special challenges with regard to controlling MRSA (or other infectious agents) because they deal with a setting considered to be the resident's own home. Residents should be able to live their normal lives regardless of MRSA colonization, but at the same time transmission of MRSA to other residents must be prevented. The most important activity is to prevent an epidemic of MRSA in LTF beforehand (MRSA-asiantuntijatyöryhmä, 2004). In an epidemic situation, MRSA-infected or -colonized residents should be isolated in private rooms or cohorted. Cohorting several MRSA-positive residents into the same room is an effective means of preventing the spread of MRSA during an outbreak of infection (Murray-Leisure et al., 1990), but its routine use limits the daily activities of residents. Intensified hand hygiene is very important in an epidemic situation. Since MRSA most commonly spreads to patients by direct contact through the contaminated hands of the nursing staff, routine practice should include cleaning hands with an antiseptic-containing solution before and after all patient contact (Bradley, 1999; Muto et al., 2003). In addition, changing gloves between residents and wearing gowns is necessary when there exists a risk of contact with blood or body fluids. The successful eradication of MRSA colonization with oral or topical (mupirocin) antibiotics has also been reported (Kotilainen et al., 2001), although efforts to control MRSA in a LTF setting have generally been incompletely effective (Cederna et al., 1990) or even unsuccessful due to a significant increase in the number of high-level mupirocin-resistant MRSA isolates (Vasquez et al., 2000). Active surveillance cultures of the residents should be done in an epidemic situation at a frequency based on the prevalence of MRSA and on risk factors for colonization (Muto et al., 2003). Surveillance cultures for MRSA should always include samples from the nostrils and, if present, from skin defects. Throat and perirectal-perineal cultures have been shown to detect *S. aureus* and MRSA with a high degree of sensitivity, but should not be selected as the only sites for a culture (Muto et al., 2003). The screening of the nostrils, the throat, and the perineum together have been reported to detect 98% of all carriers studied (Coello et al., 1994). Effectively tracking an MRSA outbreak entails keeping records of the date on which the MRSA-positive culture was performed, the site or type of infection, the contacts, the location in the facility, and antibiotic resistance patterns (Bradley, 1999; Mulligan et al., 1993). Molecular typing differentiates epidemic from nonepidemic strains, although these results may not be readily available to most nursing homes.

2.4 Laboratory diagnostics of MRSA

Reliable microbiological diagnostics of MRSA are essential for treatment, surveillance and control. Clinical microbiology laboratories play a central role in the detection, identification, antibiotic susceptibility testing, and confirmation of MRSA. Conventional laboratory detection of MRSA includes culturing the specimen with or without enrichment broth, confirmation of *S. aureus* with

identification tests, antimicrobial susceptibility testing, and finally, verification of MRSA, usually with molecular methods. This may take several days, however, and may therefore prolong unnecessary contact precautions and isolation measures in health care facilities. Rapid diagnostic testing for MRSA directly from specimens allows the infected patient to obtain a more rapid verification of antimicrobial therapy, leading to a decrease in mortality, a reduction in vancomycin usage, shorter stays in hospital, and lower hospital costs (Bootsma et al., 2006; Diekema et al., 2004b). Rapid tests are still more expensive than conventional ones, and not all laboratories are able to use them for financial reasons. In Finland, however, only a little experience with these tests currently exists. Regardless of the diagnostics methods used, concomitant cultures are necessary to recover the organism for further antimicrobial susceptibility testing and for epidemiological typing.

2.4.1 Culturing of MRSA

MRSA may be found in a culture on sheep blood agar (SBA) or other non-selective media in normal routine laboratory diagnostics. Resistance of *S. aureus* to oxacillin or cefoxitin or both in antimicrobial susceptibility testing usually leads to a suspicion of MRSA. This usually takes two to three days, and the isolate subsequently remains to be confirmed (discussed below). To achieve greater rapidity and sensitivity, screening specimens for MRSA detection are frequently cultivated onto selective media. These media contain an indicator to distinguish *S. aureus*, inhibitory agents to suppress non-staphylococcal growth, and antibiotics, usually oxacillin or cefoxitin, to select for MRSA isolates. Mannitol salt agar (MSA) or variations of this medium have been widely used as a primary isolation medium for MRSA. Its reported sensitivity and specificity have varied widely (Apfalter et al., 2002; Diederen et al., 2006; Kampf et al., 1998; Louie et al., 2006; Merlino et al., 2002a; Safdar et al., 2003; Smyth and Kahlmeter, 2005; Stoakes et al., 2006). The performance of MSA and all other selective media as well, may depend on several things. Differences in study designs may influence sensitivity and specificity; for example, some study designs test media only with pure cultures (Diederen et al., 2006; Kampf et al., 1998) while others plate swabs on several media in random (Apfalter et al., 2002) or pre-selected order (Louie et al., 2006). In addition, the salt-tolerance or antimicrobial susceptibility of a locally prevalent MRSA strain may also influence performance (Jones et al., 1997; Merlino et al., 2002b).

One of the most commonly used commercial selective media, Oxacillin Resistance Screening Agar Base (ORSAB, Hampshire, England), contains mannitol and aniline blue for the detection of mannitol fermentation, which indicates *S. aureus* growth. A high concentration of salt and lithium chloride should suppress non-staphylococcal growth, oxacillin inhibits MSSA, and polymyxin B inhibits other bacteria able to grow in such a high salt concentration. The sensitivity and specificity of ORSAB performed directly from clinical specimens for the detection of MRSA appear in Table 1. Further incubation for up to 48 h increased sensitivity, but specificity decreased. Recently, researchers have developed and evaluated a few other selective, commercial chromogenic media for accurate “next-day” detection of MRSA. Chromagar MRSA contains cefoxitin as a selective antibiotic, and due to a growth of MRSA, chromogenic substrate is hydrolyzed and mauve colonies form. In MRSA ID, MRSA forms green colonies due to the production of alpha glucosidase. The media also contain cefoxitin as a selective agent. A third cefoxitin-supplemented chromogenic agar, MRSA Select, also forms mauve colonies due to the growth of MRSA. The sensitivities and specificities of Chromagar MRSA, MRSA ID, and MRSA Select for detecting MRSA directly from patient specimens appear in Table 1. Reports indicate that at 48 h, these chromogenic media are less specific, although the sensitivity improved. The application of a cefoxitin disk on a chromogenic medium with no antibiotic has also been reported to be an attractive, alternative method of

screening for MRSA (Hedin and Fang, 2005). Based on the published data (Table 1), MRSA Select seems to be superior for MRSA screening. Care must be taken, however, in interpreting the data on sensitivity and specificity of screening media in comparative studies, because the reported performance of any medium will depend on the comparators. Hence, a medium that performs well in one study might appear less effective in another.

Enrichment media are commonly used to improve sensitivity by allowing the specimens to grow during the incubation time, usually overnight, before plating on solid media. Enrichment broths usually contain a high concentration of NaCl, 6.5% or 7.5%, recommended by the American Society for Microbiology (Isenberg, 2004), and may contain oxacillin, methicillin, cefoxitin, or ciprofloxacin or other non-beta-lactam antibiotics to add selectivity. However, high salt-containing enrichment broth has been reported to inhibit the growth of endemic MRSA strains. According to two studies, the optimal salt concentration for MRSA screening should not exceed 2.5% NaCl (Bruins et al., 2007; Jones et al., 1997), although this concentration is too low to sufficiently inhibit contaminating flora (Van Enk and Thompson, 1992). Moreover, the enrichment step introduces a delay for MRSA detection. Indicator enrichment media containing carbohydrate, indicator, and antibiotics, may be useful for detecting growth without a subculture. All the negative broths can be discarded if the sensitivity is good, while confirmatory tests are performed on isolates from only the positive broths. The use of the commercial indicator enrichment medium evaluated thus far has shown a sensitivity of 85%, but a specificity of only 43.6% (Gurran et al., 2002).

Table 1. Solid agar media used for MRSA detection in clinical specimens.

Medium (Abbreviation)	Total MRSA isolates/no. of samples	Specimens from (no.)	Comparator media or tests	Sensitivity (%) after incubation for		Specificity (%) after incubation for		Reference
				24 h	48 h	24 h	48 h	
Oxacillin Resistance Screening Agar (ORSAB)	104/455	nares (132) perineum (109) skin and soft tissue (206) sputum (5) urine (3)	mannitol-salt agar with oxacillin 2mg/L	76.0	98.0	ND[a]	89.7	(Simor et al., 2001)
Oxacillin Resistance Screening Agar (ORSAB)	236/579	nares (131) axilla (119) inguinal (70) throat (60) anal (34) insertion sites of devises (24) other (80)	Phenyl-mannitol-salt agar with oxacillin 1 µg disk Columbia blood agar with 10 µg colistin disk	50.8	68.2	95.6	94.5	(Apfalter et al., 2002)
Oxacillin Resistance Screening Agar (ORSAB)	85/747	nose (192) throat (180) axillae (209) perineum (119) wounds (47)	CHROMagar MRSA MRSA ID	62.0	78.0	97.9	93.1	(Perry et al., 2004)
Oxacillin Resistance Screening Agar (ORSAB)	30/366	perineum (153) throat (116) nose (54) mouth (30) wounds (8) umbilical region (3) ear (2)	CHROMagar MRSA MRSA ID	57	77	92	83	(Compernolle et al., 2007)
CHROMagar MRSA	85/747	nose (192) throat (180) axillae (209) perineum (119) wounds (47)	ORSAB MRSA ID	59.0	72.0	99.3	92.1	(Perry et al., 2004)
CHROMagar MRSA	146/2015	nares (2015)	Trypticase soy agar	82.2	95.2	99.7	99.7[b]	(Flayhart et al., 2005)
CHROMagar MRSA	111/2125	nares (1243) perineal (882)	MRSA*Select* Mannitol-salt agar with 6 mg/L oxacillin Mannitol-salt agar with cefoxitin	74.8	82.9	99.1	ND[a]	(Stoakes et al., 2006)
CHROMagar MRSA	113/831	nares (831)	Trypticase soy agar with blood Müller-Hinton agar with 10 mg/L tobramycin	95.6	98.3	100	100	(Loulergue et al., 2006)
CHROMagar MRSA	30/366	perineum (153) throat (116) nose (54) mouth (30) wounds (8) umbilical region (3) ear (2)	ORSAB MRSA ID	67	73	98	90	(Compernolle et al., 2007)

[a]ND means not determined, or could be calculated according to data published.
[b]By excluding mauve colonies that were coagulase negative or in consistent with MRSA in Gram staining.

Table 1. (*continued*)

Medium (Abbreviation)	Total MRSA isolates/no. of samples	Specimens from (no.)	Comparator media or tests	Sensitivity (%) after incubation for		Specificity (%) after incubation for		Reference
				24 h	48 h	24 h	48 h	
CHROMagar MRSA	78/205	nose (101) groin (52) axilla (52)	MRSA ID MRSA*Select* IDI-MRSA PCR test GenoType MRSA Direct -PCR test	63	71	99	67	(van Hal et al., 2007)
MRSA ID	85/747	nose (192) throat (180) axillae (209) perineum (119) wounds (47)	ORSAB CHROMagar MRSA	80.0	89.0	99.5	85.6	(Perry et al., 2004)
MRSA ID	30/366	perineum (153) throat (116) nose (54) mouth (30) wounds (8) umbilical region (3) ear (2)	CHROMagar MRSA ORSAB	60	77	98	94	(Compernolle et al., 2007)
MRSA ID	78/205	nose (101) groin (52) axilla (52)	CHROMagar MRSA MRSA*Select* IDI-MRSA PCR test GenoType MRSA Direct -PCR test	71	82	98	53	(van Hal et al., 2007)
MRSA*Select*	111/2125	nares (1243) perineal (882)	CHROMagar MRSA Mannitol-salt agar with 6 mg/L oxacillin Mannitol-salt agar with cefoxitin	97.3	ND[a]	99.8	ND[a]	(Stoakes et al., 2006)
MRSA*Select*	181/6199	nares (2483) perianal (2312) catheter exit site (647) skin/soft tissue (632) sputum (58) urine (67)	Mannitol-salt agar with 8 mg/L cefoxitin	93.0	98.0	99.5	92.0	(Louie et al., 2006)
MRSA*Select*	78/205	nose (101) groin (52) axilla (52)	CHROMagar MRSA MRSA ID IDI-MRSA PCR test GenoType MRSA Direct -PCR test	64	69	95	74	(van Hal et al., 2007)

2.4.2 Identification of *S. aureus*

S. aureus forms golden or white colonies on blood agar. All the strains produce catalase, an enzyme which converts hydrogen peroxide (H_2O_2) to water and oxygen. The catalase test is useful to distinguish staphylococci from enterococci and streptococci. Most of these strains ferment mannitol and trehalose anaerobically which, among other tests, may serve to identify *S. aureus*. *S. aureus* is able to clot extracellular plasma, and can thereby be differentiated from most other staphylococci (Bannerman, 2003). For routine testing, more rapid coagulase tests are used, especially commercial latex agglutination tests (Gupta et al., 1998; van Griethuysen et al., 2001). Early versions of these latex tests detected only fibrinogen binding protein (clumping factor) and protein A on the cell

surface of *S. aureus*, and problems arose with some MRSA strains (Kuusela et al., 1994). Later formulations of these tests also detected either capsular polysaccharide or other group-specific antigens, and are considered reasonably accurate, although none of these tests can correctly distinguish *S. aureus* from all other *Staphylococcus* isolates (Gupta et al., 1998; van Griethuysen et al., 2001). Deoxyribonuclease (DNase) plates can also serve to identify *S. aureus*, although some coagulase-negative staphylococci (CoNS) produce sufficient DNase to give positive results in DNase tests (Menzies, 1977). Several biochemical identification kits and automated systems are also available. Performances of these tests are reported to be good (Fonsale et al., 2004; Layer et al., 2006; Ligozzi et al., 2002; Spanu et al., 2003), although most of them are technically time consuming or expensive. Molecular tests are sometimes needed to confirm the identity of *S. aureus*. The DNA probe test for *S. aureus*-specific 16S rRNA yields results of *S. aureus* or non-*S. aureus*. This rapid test has served as a reference method for other commercial rapid tests when identifying *S. aureus* directly from blood cultures (Chapin and Musgnug, 2003). Most molecular methods are still PCR based, and a range of primers designed to amplify *S. aureus*-specific targets have been developed. Genes such as *nuc* (nuclease), 16S rRNA, *coa* (coagulase), *clfA* (clumping factor), and *femA* can distinguish *S. aureus* from other staphylococci, but *S. aureus*-specific molecular tests are usually combined with the simultaneous detection of *mec*A, thus enabling the identification of MRSA isolates (Brakstad et al., 1993; Geha et al., 1994; Kearns et al., 1999; Mason et al., 2001; Vannuffel et al., 1995).

2.4.3 Phenotypic detection of methicillin resistance in *S. aureus*

In assessing the performance of antimicrobial susceptibility testing methods, the minimum inhibitory concentration (MIC) determined by a dilution method has traditionally been the reference method. The Etest method (AB Biodisk, Solna, Sweden) also provides a MIC result, and is simpler to perform than the dilution method. The disk diffusion method is, however, the most widely used method for confirming methicillin resistance in clinical microbiology laboratories. For PBP2a detection, a rapid and commercially available latex agglutination test is commonly used (Nakatomi and Sugiyama, 1998). In this method, PBP2a is extracted from a suspension of colonies and detected by agglutination with latex particles coated with monoclonal antibodies to PBP2a. Automated methods include tests for methicillin and oxacillin susceptibility and are generally considered reliable for *S. aureus*, although a few incorrect results have been reported (Frebourg et al., 1998; Kampf et al., 1999; Ligozzi et al., 2002; Ribeiro et al., 1999). The Clinical and Laboratory Standard Institute (CLSI, formerly NCCLS; USA) is a leading committee which recommends standards for antimicrobial susceptibility testing for global application, and the Finnish national laboratory guidelines on antimicrobial susceptibility testing (FiRe-standard) follow those of the CLSI.

Several conditions used in phenotypic *in-vitro* laboratory tests, including medium, inoculum, incubation time and temperature, and test agent, are known to affect the expression of resistance (Brown, 2001; Sutherland and Rolinson, 1964). MRSA is reportedly more clearly distinguished on Müller-Hinton (MH) agar than on Iso-Sensitest, Diagnostics Sensitivity Test (DST), and PDM media (Brown and Kothari, 1974; Monsen et al., 2003), although researchers have reported variation in methicillin susceptibility test performance with MH medium from different manufacturers (Hindler and Inderlied, 1985; Mackenzie et al., 1995) and between different batches of MH medium from a single manufacturer (Coombs et al., 1996). CLSI has recommended the addition of 2% NaCl to MH only in the dilution method (CLSI, 2006), while the British Society for Antimicrobial Chemotherapy (BSAC) recommends the addition of salt for both dilution and disk diffusion methods (BSAC, 1998). Inoculum density is also an important factor for expressing

resistance. A larger inoculum increases the likelihood of detecting the heterogeneously resistant cell population, but a heavy inoculum together with an increased concentration of NaCl may also lead to increased false-resistant reports (Huang et al., 1993). Lowering the incubation temperature to 30°C (Merlino et al., 2002b), and extending the incubation time to 48 h (Brown, 2001) may improve the reliability of detection. The test agent has traditionally been methicillin or oxacillin. Recently, cefoxitin disk diffusion test has been recommended for the reliable detection of methicillin resistance (Cauwelier et al., 2004; CLSI, 2006; Felten et al., 2002; Skov et al., 2003; Skov et al., 2006; Swenson and Tenover, 2005). Cephamycins, including cefoxitin, have a high affinity for *S. aureus* PBP4, a protein involved in cell wall cross-linking. Previous experiments showed a relationship between PBP2, PBP4, and methicillin resistance (Murakami et al., 1987). Cefoxitin is assumed to be a better inducer of *mec*A gene expression than is oxacillin or methicillin, and thus, is better for screening heterogeneous MRSA populations expressing the *mec*A variable. The major advantage of cefoxitin is that the optimum test conditions are similar to those used in the susceptibility testing of other antibiotics (Skov et al., 2006).

2.4.4 Verification of MRSA with in-house molecular methods

PCR-based methods have been used routinely by reference laboratories as the standard method for detecting and verifying MRSA. Primers for the amplification of the *mecA* gene and *S. aureus*-specific gene loci such as *nuc*, 16S rRNA, *coa*, *clfA, femA*, and *femB*, have served that purpose (Brakstad et al., 1993; Geha et al., 1994; Kearns et al., 1999; Kobayashi et al., 1994; Mason et al., 2001; Vannuffel et al., 1995). A number of studies have examined the use of molecular methods for the direct detection of MRSA in positive blood cultures, including gel-based PCR (Louie et al., 2002; Maes et al., 2002) and real-time PCR (Shrestha et al., 2002; Tan et al., 2001). The detection of MRSA direct from non-sterile sites such as the nostrils, the throat, wounds and the perineum is in great demand. The development of such methods is more complex because the *mecA* gene is highly conserved among staphylococci, and primers used to detect *S. aureus* and *mecA* will give MRSA positive results when tested on a mixed culture containing an MSSA and a methicillin-resistant CoNS. Many strategies have been directed to reduce false positives. The use of MRSA-selective enrichment broth prior to duplex PCR for *mecA* and *femB* (Jonas et al., 2002), and prior to *nuc* gene amplification followed by use of *mecA* and *nuc* primers for those samples which were detected as *nuc* positive after the enrichment step by real-time PCRs (Fang and Hedin, 2003), has been developed and evaluated. In addition, an isothermal signal amplification method (CytAMP) has been applied to the detection of MRSA from screening swabs (Levi et al., 2003). This assay simultaneously targets *coa* and *mecA* genes, and the results were obtained as a colour signal on a 96-well microtiter plate within 3 h following overnight enrichment in the selective broth. Since all of these strategies require an enrichment step, neither of them can be used for same-day MRSA reporting. A rapid MRSA detection assay, which consists of an immunomagnetic enrichment step, first used to selectively capture *S. aureus* using an antibody to protein A, followed by the extraction of immunocaptured bacterial DNA and their identification by a triplex quantitative PCR for *mecA*, *S. aureus femA*, and CoNS *femA* genes (Francois et al., 2003). This assay enables the detection and identification of MRSA in less than 6 h after sample collection.

2.4.5 Commercial applications for MRSA verification

Commercial PCR-based assay GenoType® MRSA (Hain Lifescience, Germany) is a rapid, 3-h method enabling the detection of the *S. aureus*, *Staphylococcus epidermidis*, and *mecA* gene. The assay involves rapid DNA isolation and PCR amplification, followed by hybridization using specific oligonucleotide probes immobilized onto a membrane (Cuny et al., 2001). Special equipment for agarose gel electrophoresis and waste treatment for ethidium bromide is unnecessary with this assay. The EVIGENE™ MRSA Detection (AdvanDx, Inc, Woburn, MA; For Research Use Only) kit is a rapid (3-h), non-PCR assay, which detects *mecA* and *nuc* genes by colorimetric gene probe hybridization in a microwell strip format (Skov et al., 1999; Smyth et al., 2001). Both of these methods, however, require a pure culture for the detection of MRSA.

Tests detecting MRSA directly from blood cultures are also available as commercial applications. GenoType® BC Gram-positive (Hain Lifescience, Germany) enables the detection of *S. aureus* among 16 other gram-positive bacterial species and its resistance to methicillin and vancomycin within a few hours, according to the manufacturer, and in conjunction with a 3-h enrichment step for the use of the EVIGENE™ MRSA Detection kit, will provide the results within seven hours (Levi and Towner, 2003; Poulsen et al., 2003). There are a few recently developed commercial assays available which may detect MRSA directly from non-sterile sites and enable same-day reporting (Table 2). BacLite® Rapid MRSA (Bio-Stat Diagnostic System, UK) was developed to detect ciprofloxacin-resistant MRSA strains directly from nasal swabs, and measures the activity, detected by a bioluminescence system, of adenylate kinase, an essential house-keeping enzyme. The test involves selective broth enrichment, magnetic microparticle extraction, and the selective lysis of *S. aureus* to add target organism specificity, and the results are available within five hours. GenoType® MRSA Direct (Hain Lifescience, Germany) is a 4- to 6-h test for clinical swabs involving DNA extraction and PCR amplification followed by the hybridization of oligonucleotide probes onto a membrane, and detection by staining. This assay uses primers for *orfX* and one primer pare for a unique region of SCC*mec*. IDI-MRSA™ (Infectio diagnostic Ste-Foy, Quebec) is a 1- to 2-h assay involving the extraction of DNA directly from clinical nasal swabs followed by one-step real-time PCR. For amplification, five forward primers targeting the SCC*mec* right extremity regions (SRE) corresponding to SCC*mec* types I, II, III, IVa, IVb, and IVc are combined with one reverse primer and three molecular beacons specific to the *orfX* region (Huletsky et al., 2004). False negative results may be due to a polymorphism of SRE, since the assay has limited numbers of primers for SRE. In addition, some MRSA strains may lose the *mec*A gene while maintaining fragments of the SCC*mec* gene, thus resulting in false positive results (Donnio et al., 2005).

The relative sensitivities and specificities of the IDI-MRSA™ and GenoType® MRSA Direct assays have been compared and according to these studies, the IDI-MRSA™ seemed to have better performance (Francois et al., 2007; van Hal et al., 2007) and is more cost-effective (van Hal et al., 2007). One possible reasons for the lower sensitivity of GenoType® MRSA Direct than that of IDI-MRSA™ may be related to the difference in the detection limits of the assays: with IDI-MRSA™, detection limit is 25 CFU per nasal swab, and with GenoType® MRSA Direct, 30 CFU per 5 µl. Since each IDI-MRSA™ run could be completed in 2 h with a Smart Cycler DNA amplification system containing 16 random access reaction places, 64 specimens could be detected within working hours a day (reaction places for positive and negative controls are needed in each run). The GenoType® MRSA Direct assay run takes 4-6 h to obtain final results, and thus, using 12-places incubator (TwinCubator®) at most 24 specimens could be detected a day. In 2007, the Xpert™ MRSA system from the same manufacturer than IDI-MRSA™ came to the market. Thus far, it is the only available system that combines sample preparation with real-time PCR amplification and

detection for fully integrated and automated nucleic acid analysis. The system enables users to perform up to 16 different molecular tests concurrently in an hour. Hence, with the Xpert™ MRSA system, total of 128 specimens could be detected a day. Sensitivity, specificity, positive predictive value, and negative predictive value of the assay of 86.3%, 94.9%, 84.5%, and 96.9%, respectively, has been reported by the manufacturer.

Table 2. Evaluated commercial tests for MRSA screening directly from clinical specimens.

Test	Total MRSA isolates/no. of samples	Specimens from (no.)	Comparator test	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %	Reference
BacLite® Rapid MRSA	157/1382	nostrils (1382)	culture with mannitol-salt agar	90.4	95.7	72.8	98.7	(Johnson et al., 2006)
GenoType® MRSA Direct	37/508	nose (209) throat (101) skin (80) groin (46) axilla (12) wound (34) other (26)	enrichment culture with thioglycolate broth, culture with Columbia-CNA-agar supplemented with colistin and nalidixic acid	94.6	98.7	85.4	99.6	(Holfelder et al., 2006)
GenoType® MRSA Direct	93/182	strain collection of clinical isolates: MRSA (93) MSSA (89)	culture (not specified), conventional PCR, IDI-MRSA™	67[a] 90[b]	57[a] 53[b]	61[a] 66[b]	63[a] 84[b]	(Francois et al., 2007)
GenoType® MRSA Direct	78/205	nose (101) groin (52) axilla (52)	culture with CHROMagar MRSA, MRSA ID, and MRSA*Select*, IDI-MRSA™	69	96	92	82	(van Hal et al., 2007)
IDI-MRSA™	72/288	nostrils (288)	culture with mannitol-salt agar, enrichment culture with tryptic soy broth	91.7	93.5	82.5	97.1	(Warren et al., 2004)
IDI-MRSA™	81/331	nostrils (331)	culture with mannitol-salt agar	100	98.4	95.3	100	(Huletsky et al., 2005)

[a]Positive signal was recorded only for bands showing intensities higher that of the control
[b]Positive results were recorded onto the strip for any band corresponding to the MRSA location, whatever its intensity

Table 2. (*continued*)

Test	Total MRSA isolates/no. of samples	Specimens from (no.)	Comparator test	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %	Reference
IDI-MRSA™	74/287	pooled nasal and rectal swabs (174) nasal (53) rectal (28) other (32)	enrichment culture with brain heart infusion broth supplemented with 5 mg/L aztreonam and 75 mg/L ceftizoxime	96	96	90	98	(Desjardins et al., 2006)
IDI-MRSA™	93/182	strain collection of clinical isolates: MRSA (93) MSSA (89)	culture (not specified), conventional PCR, GenoType® MRSA Direct	94	64	71	92	(Francois et al., 2007)
IDI-MRSA™	100/997	nose (522) throat (212) perineum (206) skin wounds (60)	culture with MRSA-ID, enrichment culture with brain heart infusion broth	81.0	97.0	75.0	97.9	(de San et al., 2007)
IDI-MRSA™	78/205	nose (101) groin (52) axilla (52)	culture with CHROMagar MRSA, MRSA ID, and MRSA*Select*, GenoType® MRSA Direct	90	96	93	94	(van Hal et al., 2007)

2.5 Evolution of MRSA

2.5.1 Methods for investigating the evolution of MRSA

Monitoring the spread of MRSA strains requires efficient and accurate epidemiologic typing systems that allow the recognition of isolates originated from a common ancestor and the discrimination between unrelated isolates. An optimal typing method should show high typeability (*i.e.* referring to the ability of the test to provide unambiguous results for each isolate examined), high reproducibility (*i.e.* referring to the ability of the technique to produce the same result with repeated tests), high discriminatory power, and stability. In addition, ease of use and ease of interpretation, rapidity, and low cost may also be criteria affecting the choice of method (Struelens, 1996; van Belkum et al., 2001).

Phenotypic methods such as phage typing and antimicrobial susceptibility testing have long been used for the discrimination of MRSA strains (Parker, 1972; Rossney et al., 1994). Phage typing is based on lysis to a standard set of phage. Antimicrobial susceptibility testing usually produces the first typing results available, and may therefore be useful in short outbreaks caused by multiresistant strains. Phenotypic typing methods, however, do not directly characterize the expression of different genes, thus environmental factors such as the antimicrobial treatment of the host may have an influence on bacterial phenotype. In addition, genetically distinct strains may share a common resistance pattern, especially multiresistant MRSA clones. However, due to the limited reproducibility and lack of typeability or discriminatory power of some of these methods, they are inadequate approaches for bacterial comparisons.

Genotypic typing methods are based on the analysis of chromosomal or extrachromosomal DNA. Plasmid analysis was the first DNA-based method to be applied to *S. aureus*, and has served in several outbreak investigations (Archer and Mayhall, 1983; Hartstein et al., 1989). However, the reproducibility of the method has been shown to be only moderate (Tenover et al., 1994), and the relative stability of staphylococcal plasmids has been debated. Restriction fragment length polymorphism (RFLP) with a variety of DNA and RNA probes has also been used to type bacterial strains, thus providing a discriminative fingerprint of the entire chromosome. In this method, chromosomal DNA is restricted by frequently cutting enzymes, followed by agarose gel electrophoresis, Southern blotting (transferring the DNA fragments from the agarose gel to a nylon membrane), and hybridization with specific probes. Ribotyping is the most widely used adequate procedure of this technique (Blumberg et al., 1992), but it has shown low discriminatory power (Tenover et al., 1994). In addition, several polymerase chain reaction (PCR) -based methods have been developed. In those methods, specific genes with variable repeat regions, such as *coa* and *spa* (Frenay et al., 1994; Hoefnagels-Schuermans et al., 1997; Tenover et al., 1994) or polymorphic non-coding repetitive sequences dispersed around bacterial genomes, serve as targets for PCR amplification (Del Vecchio et al., 1995).

SCC*mec*-typing is used for amplifying the *ccr* and *mec* gene complexes (Okuma et al., 2002). Multiplex-PCR strategies have been developed to distinguish between SCC*mec* types I-IV and some of its variants (IA, IIIA, and IIIB) (Oliveira and de Lencastre, 2002), and more recently, SCC*mec* types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V (Zhang et al., 2005). Neither of these methods, however, discriminate between the seven variants (IIA, IIB, IIC, IID, IIE, IVE, IVF) recently described (Shore et al., 2005). The discrimination power is low in SCC*mec*-typing, since only five major SCC*mec* types have been recognized thus far. SCC*mec* types may still be important in epidemiological studies.

Pulsed-field gel electrophoresis (PFGE) has been the most commonly used genotyping method for MRSA outbreak investigation and has been regarded as the gold standard method (Roberts et al., 1998; Struelens et al., 1993; Tenover et al., 1994). PFGE was introduced in 1984 (Schwartz and Cantor, 1984) and is based on RFLP banding patterns on agarose gels obtained by the digestion of chromosomal DNA by rare-cutting restriction enzymes, usually *SmaI*. This digestion generates fragments 20-800kb in size separated by an apparatus that switches the direction of the current according to a predetermined pattern. Random genetic events, including point mutations and insertions and deletions of DNA, usually alter the PFGE pattern during the course of an outbreak. The interpretation of DNA fragment patterns generated by PFGE can be assigned to one of four categories: 1) indistinguishable from the outbreak pattern, 2) closely related to the outbreak pattern, 3) possibly related to the outbreakpattern, or 4) unrelated to the outbreak pattern (Tenover et al., 1995). Comparison of restriction patterns may, however, be subjective due to the large number of fragments. In addition, PFGE is a time-consuming and laborious method, and its applicability to

evolutionary analysis is limited. Moreover, the quality of gel patterns varies, rendering the comparison of strain analyses between laboratories difficult. Recently, PFGE protocols have been harmonized in an attempt to avoid this problem (Murchan et al., 2003).

Multilocus sequence typing (MLST) is based on the sequencing of seven genes in the bacterial chromosome that are thought to be conservative. MLST has been developed for the identification of the hypervirulent lineages of *Neisseria meningitidis*, and has also served in investigating hypervirulence and antibiotic resistance among *Streptococcus pneumoniae* (Enright et al., 1999; Enright and Spratt, 1998; Maiden et al., 1998; Shi et al., 1998). Seven housekeeping genes (genes which are expressed in all cells and which code for molecules that are necessary for basic maintenance and essential cellular functions) chosen in a scheme of *S. aureus*, named *arc* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol kinase), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase), and *yqiL* (acetyl coenzyme A acetyltransferase), are widely separated around the genome, thereby minimizing the impact of recent DNA exchange (Enright et al., 2000). Isolates are defined by the alleles present at the seven loci, each of which receives numbers from various databases that are then combined with a seven-digit allelic profile. Each unique allelic profile is assigned as an ST (sequence type) and compared to other isolates online (http://www.mlst.net). Isolates with the same ST are considered members of a single clone. As a technique, however, MLST is expensive and laborious, and thus may be unsuitable for routine typing. eBURST (Based Upon Related Sequence Typing) is an algorithm used to illustrate the genetic relationship between large MLST datasets that bear some pre-defined level of similarity between allelic profiles, and to predict the ancestral genotype of each group. A dataset of ST numbers and allelic profiles are entered into the BURST program (http://www.mlst.net/BURST/burst.htm), which then sub-divides isolates sharing high genetic similarity into clonal complexes (CCs) (Feil et al., 2004).

In *spa*-typing, a polymorphic X-region of protein A is sequenced, and clustering of clonal complexes of related *spa* types are analyzed with an Internet-based tool (http://www.ridom.de/spaserver/) (Harmsen et al., 2003). The discriminatory power and concordance of *spa* typing has been reported to exist between PFGE and MLST (Hallin et al., 2007; Strommenger et al., 2006).

2.5.2 Evolutionary history of MRSA

Genetic evolutionary studies have demonstrated that MRSA has a largely clonal structure due to the low frequency of recombination between *Staphylococcus*. Investigators have suggested that point mutations are 15 times more likely to occur than recombination (Feil et al., 2003). Molecular typing techniques have disproved a theory proposed earlier that *S. aureus* acquired the *mecA* gene only once (Enright et al., 2002). Researchers have predicted that the extant ancestral MRSA is derived from MSSA by the acquisition of SCC*mec* type I and a single point mutation in one of the seven housekeeping genes (Enright et al., 2002; Robinson and Enright, 2003). The *mecA* gene has been shown to transfer into MSSA at least 20 times, since only a few successful phylogenetically distinct lineages have been found worldwide (Crisostomo et al., 2001; Deresinski, 2005; Enright et al., 2002; Oliveira et al., 2002; Robinson and Enright, 2003). The creation of an international surveillance system with harmonized methodologies was required to aid in hospital infection prevention and control. CEM/NET (The Center for Molecular Epidemiology and International Network) was created in 1995 to detect the reservoirs of major Gram-positive pathogen clones (Aires de Sousa and de Lencastre, 2004), and the HARMONY network was established as a

collection of European epidemic and other important MRSA strains and developed a standardized PFGE protocol (Murchan et al., 2003).

The distinct pandemic lineages have been named Iberian, Brazilian, Hungarian, New York/Japan, Pediatric, and EMRSA-16 clones, and the identification of clones was based primarily on a combination of PFGE, PCR-based techniques such as RAPD, Southern hybridization analysis such as ribotyping, and plasmid analysis (Aires de Sousa and de Lencastre, 2004). The Iberian clone caused a massive outbreak in 1989 in a Spanish hospital, where it was first identified (Oliveira et al., 2001). However, it seemed already to have existed in Belgium and France as early as in 1984 (Deplano et al., 2000). Subsequently, the Iberian clone was detected in many other European countries and in a hospital in New York (Krzyszton-Russjan et al., 2002; Murchan et al., 2003; Oliveira et al., 2001; Salmenlinna et al., 2002). In Finland, the clone (FIN-1) was first detected in the western part of the country in 1991 (Salmenlinna et al., 2000). The Brazilian clone was widely disseminated in Brazilian hospitals and has spread to other countries in South America (Aires de Sousa et al., 2001; Oliveira et al., 2001; Teixeira et al., 1995). It was also found in many countries in Europe (Aires de Sousa and de Lencastre, 2003; Enright et al., 2002), and was first isolated in Central Finland in 1992 (FIN-2) (Salmenlinna et al., 2002). The Hungarian clone has been widely spread in Hungarian hospitals since 1993 (Oliveira et al., 2001), and was also found to be the major clone in Taiwan and China in 1998-1999 (Aires de Sousa et al., 2003). The Hungarian clone (FIN-2c and FIN-13) was detected in Southern and Western Finland since 1998, but only 13 isolates have been found thus far (unpublished data). The New York/Japan clone was detected as the major clone in the USA, in many hospitals across Canada, in Tokyo, and in many countries in Europe (Aires de Sousa et al., 2000; Enright et al., 2002; McDougal et al., 2003; Oliveira et al., 2001; Simor et al., 2002). In Finland, the New York/Japan clone (FIN-3) was first detected in 1992 in Southern Finland (Salmenlinna et al., 2000). The Pediatric clone was reported for the first time in 1991 in a pediatric hospital in Portugal (Oliveira et al., 2001). Afterwards it was found in several regions in Europe, the USA, and Latin America (Aires de Sousa et al., 1998; Enright et al., 2002; Gomes et al., 2001; Oliveira et al., 2001). The representative strain (FIN-8) of the Pediatric clone was found in 1994 in Southern Finland (Salmenlinna et al., 2000), but since then the clone seems to have disappeared (unpublished). UK EMRSA-16 is one of the major clones found in UK hospitals (Moore and Lindsay, 2002), and has disseminated throughout many regions in Europe, Mexico, and Canada (Aires de Sousa et al., 2001; Murchan et al., 2003; Simor et al., 2002). In Finland, UK EMRSA-16 (FIN-5a) was first detected in 1995 (Salmenlinna et al., 2000), and was found only in persons having had hospital contact (Salmenlinna et al., 2002). In addition to these six major clones, studies have described several other MRSA clones, such as UK EMRSA-15, Berlin, Hannover, South German, Irish-1, and UK EMRSA-1, -2, -3, -4, -5, -6, -7, -10, -11, 12, -13, -14, and -17 (Enright et al., 2002; Witte, 1999).

MLST has, however, revealed that several MRSA clones considered distinct with PFGE and other molecular typing techniques were, in fact, indistinguishable (Enright et al., 2002) (Table 3). For example, of the major pandemic clones, the Brazilian and Hungarian clones showed ST239, and the New York/Japan and Pediatric clones showed ST5, whereas of the minor clones EMRSA-2, -6, -7, -12, -13, -14 and the Irish-1 clones showed ST8. Using MLST and eBURST, the five most common pandemic MRSA lineages can be visualized as clonal complexes: CC5, CC8, CC22, CC30, and CC45 (Enright et al., 2002). All of these lineages have acquired SCC*mec* several times, with the possible exception of CC22. Thus, some clones may differ in SCC*mec* though they possess identical ST. For example, the Brazilian clone possesses SCC*mec* type IIIA whereas the Hungarian clones possess SCC*mec* type III, and the New York/Japan clones possess SCC*mec* II whereas Pediatric clones possess SCC*mec* type IV. Nowadays, MLST is wide-spread among research and diagnostic laboratories, enabling comparison of the results obtained in different laboratories via the

Internet. SCC*mec* typing has recently been combined with MLST for MRSA typing, and therefore, MRSA findings reported in epidemiological studies are usually defined by both their sequence type (ST) and their SCC*mec* type, for example ST5-II.

Table 3. The most common pandemic MRSA lineages and their distribution. The most common names of the major clones appear in bold. The data were collected from the references in the text.

Clonal Complex (CC)	**Common name of clone**	**Sequence type of MLST and the most common SCC*mec* type**	**Data reported (for example):**
CC5	**New York/Japan**, USA100, CMRSA-2	ST5-II	Finland, Canada, Ireland, Japan, USA
	Pediatric, USA800	ST5-IV	Argentina, Colombia, Finland, France, Poland, Portugal, UK, USA
	UK EMRSA-3	ST5-I	Poland, Slovenia
	Southern Germany, Rome	ST228-I	Finland, Germany, Slovenia, Italy
CC8	UK EMRSA-2, -6, USA300, USA500	ST8-IV	Finland, France, USA
	Irish, USA500	ST8-II	Ireland, UK, USA
	Brazilian, Portugese, Vienna, Asia, UK EMRSA-1, -4, -11, CMRSA-3, **Hungarian**	ST239-III	Argentina, Brazil, Canada, Chile, China, Czech Republic, Finland, Germany, Greece, Ireland, Netherlands, Poland, Portugal, Slovenia, Sweden, Taiwan, UK, Uruguay, USA
	Iberian, UK EMRSA-5, -7	ST247-I	Belgium, Czech Republic, Finland, France, Germany, Italy, Netherlands, Poland, Portugal, Slovenia, Spain, Sweden, UK, USA
	Hannover, UK EMRSA-10	ST254-IV	Germany, UK
	Archaic (first) MRSA, UK EMRSA-8	ST250-I	Denmark, Germany, Switzerland, Uganda, UK, USA
CC22	UK EMRSA-15, Barnim	ST22-IV	Finland, Germany, Ireland, Sweden, UK
CC30	**UK EMRSA-16**, USA200, CMRSA-4	ST36-II	Belgium, Canada, Denmark, Finland, Greece, Mexico, Sweden, Switzerland, UK, USA
CC45	Berlin, USA600	ST45-IV	Belgium, Germany, Finland, Sweden, USA
	USA600, CMRSA-1	ST45-II	Canada, USA

3. AIMS OF THE STUDY

The purpose of this study was to investigate recent trends in the epidemiology and molecular epidemiology of MRSA in Finland with constantly increasing numbers of MRSA. The methods used in screening for *S. aureus*/MRSA, and the phenotypic and genotypic methods used to detect MRSA were evaluated.

The specific aims of this study were

1. To analyse the data on MRSA cases notified to the NIDR and antibiotic resistance profiles of MRSA isolates, to assess the situation in LTFs, and to review the methods used in laboratory diagnostics in Finland.

2. To examine the molecular epidemiology of MRSA in Finland in comparison to European and pandemic MRSA clones by using the nationwide collection of MRSA strains and to analyze the background information of MRSA-positive persons.

3. To perform a point-prevalence survey in a LTF after the outbreak of an MRSA strain not previously encountered in Finland, to assess the molecular epidemiology of MRSA and MSSA strains and to compare the results to the national strain collection, and to perform MRSA screening to detect low-level oxacillin-resistant isolates.

4. To assess the rate of MSSA and MRSA colonization in the largest NH in the Helsinki metropolitan area as well as the role of screening sites along with broth enrichment culture on sensitivity to detect *S. aureus*.

5. To compare phenotypic disk diffusion and MIC methods with two agents for MRSA screening, and to compare genotypic in-house PCR methods to commercial genotypic methods for verifying MRSA.

4. MATERIAL AND METHODS

A short summary of the materials and methods used in the separate studies is presented in Table 4.

Table 4. Specimens, selection criteria, and methods used in the study. The text contains a more detailed description.

Study	Specimens	Selection criteria (number of specimens or isolates studied)	Methods used[a]
I	Isolated strains	All MRSA isolates in Finland from the years 1997-2002, one per person (1718 [NIDR] and 1702 [NRL])	- Data analysis of antimicrobial susceptibilities from NRL - Data analysis of MRSA and invasive *S. aureus* cases from NIDR - Analysis of the methods for MRSA identification and screening activity at the national level with an emailed questionnaire –based survey - Analysis of the MRSA situation in nursing homes with a questionnaire –based survey
II	Isolated strains	All MRSA isolates in Finland from the years 1997-2004, one per person (4026 [NIDR] and 4091 [NRL])	- Data analysis of MRSA and invasive *S. aureus* cases from NIDR - Analysis of the background information on MRSA-positive persons - PFGE - MLST - SCC*mec* by in-house PCR - PVL by in-house PCR
III	Screening specimens from nostrils, skin lesions, and catheter urines	From all long term facility residents present and volunteered in the Northern Finland (90 specimens, 30 isolates)	- Description of nursing home setting - Sampling - Culture of screening specimens - Conventional biochemical methods - Antimicrobial susceptibility - GenoType® MRSA test - PVL by GenoType® Staphyloccus test - PFGE - MLST - SCC*mec* by in-house PCR
IV	Screening specimens from nostrils, throat, perineum, skin lesions, catheter exit sites, and catheter urines	From all nursing home residents present and volunteered in the Helsinki metropolitan area (663 specimens, 165 isolates)	- Description of nursing home setting - Sampling - Culture of screening specimens - Conventional microbiological methods - Antimicrobial susceptibility - GenoType® MRSA test - PFGE
V	Isolated strains, reference strains	A: Consecutive isolates from March-April 2004 (122) B: Control strains of *S. aureus*, including Finnish epidemic MRSA isolates (42) C: Control strains of other species and genera including clinical and screening isolations of *Staphylococcus epidermidis*, and reference strains of different CoNS and enterococci (62)	- Antimicrobial susceptibility for A and B - In-house PCR for A and B - GenoType® MRSA-test for A, B and C - EVIGENE™ MRSA Detection for A and B

Studies I, II, and V include *S. aureus* identification, antimicrobial susceptibility testing, *mec*A verification by PCR, MLST and SCC*mec* typing with applicable parts in a routine typing scheme at the KTL: these methods, however, were not necessarily the methods used in the specific study.

4.1 National MRSA surveillance and bacterial strain collection (studies I, II, and V)

Finnish clinical microbiology laboratories notify all MRSA isolations to the NIDR at the KTL. The date, source of specimen, and the patient's birth date, gender, and health care institution are recorded. Only one notification per person is registered per 36-month period. In case of blood or cerebrospinal fluid (CSF) specimens, the time interval is three months. Studies I and II used NIDR data. Clinical microbiology laboratories send all MRSA isolates to the NRL at the KTL for verification of oxacillin-resistance and typing (see 4.4.4-4.4.5 and 4.5.1-4.5.3). The *S. aureus* collection includes the Harmony collection (Murchan et al., 2003) in addition to all Finnish isolates sent by clinical microbiology laboratories. Studies I, II, and V used NRL data.

In study V, a total of 226 bacterial isolates were studied, including 122 consecutive clinical *S. aureus* strains sent to the KTL for verification and typing in April-May 2004. Other *S. aureus* isolates studied included 40 Finnish epidemic MRSA strains representing 24 different *Sma I* macrorestriction patterns (Tenover et al., 1995), and 2 low-level oxacillin-resistant MRSA strains. Other species and genera used in this study included 40 clinical *Staphylococcus epidermidis* strains from invasive or other sites from the Helsinki University Hospital and from a LTF outside the Helsinki metropolitan area, 2 ATCC reference *Staphylococcus epidermidis* strains, 18 other ATCC staphylococcal reference strains, 1 *Enterococcus faecalis* isolated from the subclavia catheter, and 1 *E. faecium* isolated from feces, both used as the reference strains in vancomycin-resistant enterococci (VRE) diagnostics in the NRL. One isolate per person was studied.

4.2 Definitions and nomenclature of strains (studies I-V)

In study I, multi-drug resistance (MDR) and non-multi-drug resistance (NMDR) were defined as three or more antibiotic groups other than beta-lactams, and two or fewer antibiotic groups, respectively, being ineffective against the MRSA strains according to CLSI (formerly NCCLS). Strain types were identified by PFGE (studies II-V), SCC*mec* typing (studies II and III), and MLST (studies II and III). PFGE served as the main method for typing, and one representative strain of each PFGE type was typed with SCC*mec* and MLST. Antibiotic resistance typing served as a supportive method along with molecular methods.

Isolates differing by seven or more bands with PFGE were considered different types (sporadic), and those differing by one to six bands were considered subtypes (Tenover et al., 1995). The *S. aureus* PFGE database at the KTL serves as the basis for naming the isolates (after naming the isolates, they are called strains or strain types). The database includes all Finnish epidemic MRSA strains and the Harmony collection. If the PFGE profile represents a previously recognized Harmony collection profile, the isolate has been named accordingly. Otherwise, the name has been assigned based on the site of the outbreak or the name of the hospital. Since 2005, all MRSA strain names have become numeric. Subtypes are additionally labelled with letters. Sporadic strains were defined as those found from only one person during the study period 1997-2004. Outbreak/epidemic strains of MRSA were isolated from more than one person. For the analysis, only one strain per person was included, except with persons showing two or more different strains.

4.3 Epidemiological background information

4.3.1 Questionnaire-based surveys (studies I and II)

In study I, an email was sent to the largest clinical microbiology laboratories (n=23), which are members of the Finnish Study Group for Antimicrobial Resistance (FiRe), to ask about their MRSA screening methods. The questions on their laboratory methods included: 1) the composition of media used for antimicrobial susceptibility testing (MH or other), 2) the percentage of salt added, 3) the incubation temperature and time, 4) the usage of MRSA-selective agar media, 5) the usage of a latex agglutination test for detection of PBP2a, and 6) PCR-based detection of *mecA*-gene. To assess the screening activity of hospital districts, the number of MRSA cultures performed and the number of MRSA strains isolated in 2001 was also requested from the laboratories.

The MRSA situation in long-term facilities in 2001 was assessed in study I with a questionnaire sent by email to the infection control nurses of each Finnish hospital district (n=21). The nurses were asked whether MRSA was found in patients or staff members of long-term facilities located in their hospital district. The types of long-term facilities involved and the number of MRSA findings were also inquired.

In study II, additional background information from 2001 to 2003 were collected from persons who tested positive for MRSA by sending questionnaires to infection control nurses at relevant health care institutions. The information collected included 1) whether the MRSA positive person was a patient or a staff member; 2) whether the specimen was taken on clinical or screening basis; 3) whether the patient was screened because of a hospital contact abroad (hospitalization or surgery outside the Nordic countries in the six months prior to testing) or because of exposure to MRSA, and whether the staff member was screened because of a hospital contact abroad (worked outside the Nordic countries in the six months prior to testing) or because of an epidemic situation, and 4) whether the specimen was taken for another reason.

4.3.2 Settings and outbreak in long-term facilities (studies III and IV)

In study III, an MRSA outbreak in a 34-bed health care ward (HCW) and an associated 46-bed NH with 76 participating residents was described in a small municipality of 5000 inhabitants in Northern Finland. Patient transfers from the HCW to the nearby secondary care hospital occurred up to approximately twice per week, from the NH to the HCW, approximately 40 times per year, and to the secondary care hospital, between two to five times per year. Patients included mainly the elderly with multiple diseases. The first isolate of MRSA was found in a urine culture in a 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 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 2003; 12 new patients tested positive for MRSA, all from LTFs. In total, 726 clinical bacterial cultures were examined in the entire municipality in 2003. None tested positive for MRSA, except the urine culture of the index patient. A new PFGE profile (FIN-22) was obtained from all the 13 isolates of the different patients. The point-prevalence study was carried out on 26 February 2004. Demographic data (age, sex, antimicrobials, foreign devices, and length of nursing stay) for each patient were collected and presented in the original publication.

Study IV was performed in the largest NH (25 wards with a total of 584 beds) in the Helsinki metropolitan area. In 2004, a total of 685 long-term residents (mean length of stay; 3.1 years) and 420 short-term residents (approximately 1060 short-term nursing periods) were treated in the NH (annual occupancy; 99.3%). Of the residents, 76% were females with a mean age of 83 years, 62% of whom suffered from dementia. Of the 25 wards, 9 were selected based on the following criteria: 1) at least one of the residents had either an indwelling catheter or open skin lesions and 2) there were no previously known MRSA patients. Of the study residents, 4% were bedridden. The average annual turnover among the long-term residents of the study wards was five, and among the short-term residents 100. The study was carried out in each ward in one day during the week of 27 September – 1 October 2004.

4.4 Laboratory diagnostics of *S. aureus* and MRSA

4.4.1 Sampling (studies III, and IV)

All residents present volunteered to have their nostrils and skin lesions swabbed, and catheter urines cultured for studies III and IV; additionally, throats, perineal sites, and catheter exit sites were swabbed for study IV.

4.4.2 Culturing (studies III, IV, and V)

The screening swabs (Probact transport swab; Schofield St-Heywood, U.K.) were cultured onto the salt-containing (6.5% NaCl) enrichment trypticase soy broth (TSB) and then onto the non-selective SBA as a control plate for TSB in study IV, and directly onto the SBA and the ORSAB (CM1008, Oxoid, Hampshire, England) plates selectively supplemented with 2 mg/l of oxacillin, and 100 000 U/l of polymyxin B (Oxoid) in study III at the KTL (Figure 2). Perineal and catheter exit site swabs, and catheter urines were also cultured onto the non-selective cystine lactose electrolyte-deficient (CLED) agar to prevent the possible overgrowth of *Proteus* sp. (study IV). The SBA and CLED agar plates, and TSB tubes were incubated aerobically at +36 (±1)°C for 24 h, and on the next day, were recultured from the TSB onto the SBA and the ORSAB (study IV). The SBA plates were incubated aerobically at +36 (±1) °C for 48 h, and the ORSAB plates for 96 h; and both were inspected daily (studies III and IV). The agar plates cultured on day one (SBA and CLED) were incubated aerobically at +36 (±1) °C for an additional 24 h (study IV). *S. aureus*-like colonies from the SBA and CLED, and blue colonies indicating oxacillin-resistant *S. aureus* from the ORSAB, were selected (one to three *S. aureus* colonies per specimen), subcultured onto the SBA (in the case of swarming *Proteus* sp., subcultures were also placed onto CLED in study IV), and the pure cultures were incubated overnight at +36 (±1)°C (studies III and IV).

In study V, the control strains were revived from frozen stocks (-70°C), subcultured on sheep blood agar and incubated overnight at +37°C. The suspected MRSA strains sent by clinical laboratories were cultured like frozen stocks if received in rayon swabs.

4.4.3 Identification (studies I-V)

S. aureus species identification was verified with Slidex agglutination test (bioMérieux, Marcy l´Étoile, France) in studies I, II, and V. Additional tests included *nuc*-PCR [Brakstad, 1992], the tube coagulase test, API Staph (BioMérieux, Marcy l´Étoile, France), API ID 32 Staph (BioMérieux, Marcy l´Étoile, France), and other microbiological fermentation tests routinely used in the NRL. Since the year 2000, all isolates have been tested routinely for correct species identification and oxacillin resistance with *nuc*- and *mec*A-PCRs, respectively (see 4.4.5). Microbiological fermentation tests were performed to verify *S. aureus* species only if the *nuc*-gene was absent. In studies III and IV, *S. aureus* was detected with DNAse production, the fermentation of mannitole and trehalose, and the Slidex agglutination test (bioMérieux, Marcy l´Étoile, France) (Figure 2). In study IV, the tube coagulase test was used on all *S. aureus* strains found, or as an additional test, if needed, in study III. Clinical *Staphylococcus epidermidis* isolates used in study V were identified with conventional microbiological fermentation tests.

4.4.4 Antimicrobial susceptibility testing (studies I-V)

Oxacillin resistance was determined with disk diffusion methods based on CLSI recommendation using Oxoid disks (Oxoid, Hampshire, England) for all isolates sent to the KTL for further analysis (studies I and II), including 164 *S. aureus* isolates (the 122 clinical isolates sent for *mecA* confirmation, and 42 control strains) in study V, and all *S. aureus* isolates found (one *S. aureus* colony per specimen) in studies III and IV (Figure 2). The cefoxitin disk test was also used to determine oxacillin resistance in studies IV and V. The MIC test for oxacillin was used for all isolates sent to the KTL for further analysis (studies I, II, and V), and for all *S. aureus* isolates (study III); the E-test was used according to the manufacturer's instructions (AB Biodisk, Solna, Sweden) for isolates expressing disk diameters of oxacillin under 13 mm or of cefoxitin under 20 mm (study IV). Additional resistance to erythromycin, clindamycin, gentamicin, tobramycin, ciprofloxacin (studies I, II, and IV), levofloxacin (study III), rifampin, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin, tetracycline, fusidic acid, linezolid (study III), mupirocin, and chloramphenicol (Oxoid, Hampshire, England) was tested with the disk diffusion method for all isolates (studies I, II, and III), or only in case of MRSA (study IV). Antimicrobial tests were performed in MH agar plate containing either 2% NaCl with the oxacillin disk diffusion test (studies I, II, and V) and E-test (studies I-V), or without the addition of salt (study V). With the cefoxitin disk test, the plates were incubated either with (study V) or without the addition of salt (studies IV and V). The plates with antimicrobial disks were incubated at +35°C, and with the E-test, at +30°C for 24 h (studies III, IV, and V) or overnight (studies I and II). In study V, both of these temperatures were used to determine oxacillin and cefoxitin resistance. Resistance and sensitivity for antimicrobials were interpreted according to CLSI critical diameters.

4.4.5 Verification of MRSA (studies I -V) and detection of Panton-Valentine leukocidin (PVL) genes (studies II and III)

S. aureus species identification and oxacillin resistance were tested as described earlier with in-house *nuc* (Brakstad et al., 1992)- and *mec*A (Murakami et al., 1991) -PCRs in studies I, II and V. DNA isolation with guanidium thiocyanate (Pitcher, 1989) preceded PCR. All biochemically identified *S. aureus* strains in study III, or *S. aureus* strains expressing oxacillin MIC in excess of two in study IV, and all 232 bacterial isolates in study V, were checked for *mec*A- and *S. aureus*-

specific fragments in duplicates by multiplex PCR reaction with the GenoType® MRSA test (Hain Lifesciences, Germany) using the reagents supplied according to the manufacturer's instructions. The EVIGENE™ MRSA Detection kit was used according to manufacturer's instructions to identify 232 bacterial isolates in study V. PVL was determined with the GenoType® *Staphylococcus* test (study III) (Hain Lifesciences, Germany) according to the manufacturers' instructions, or with in-house PCR (study II) (Lina et al., 1999).

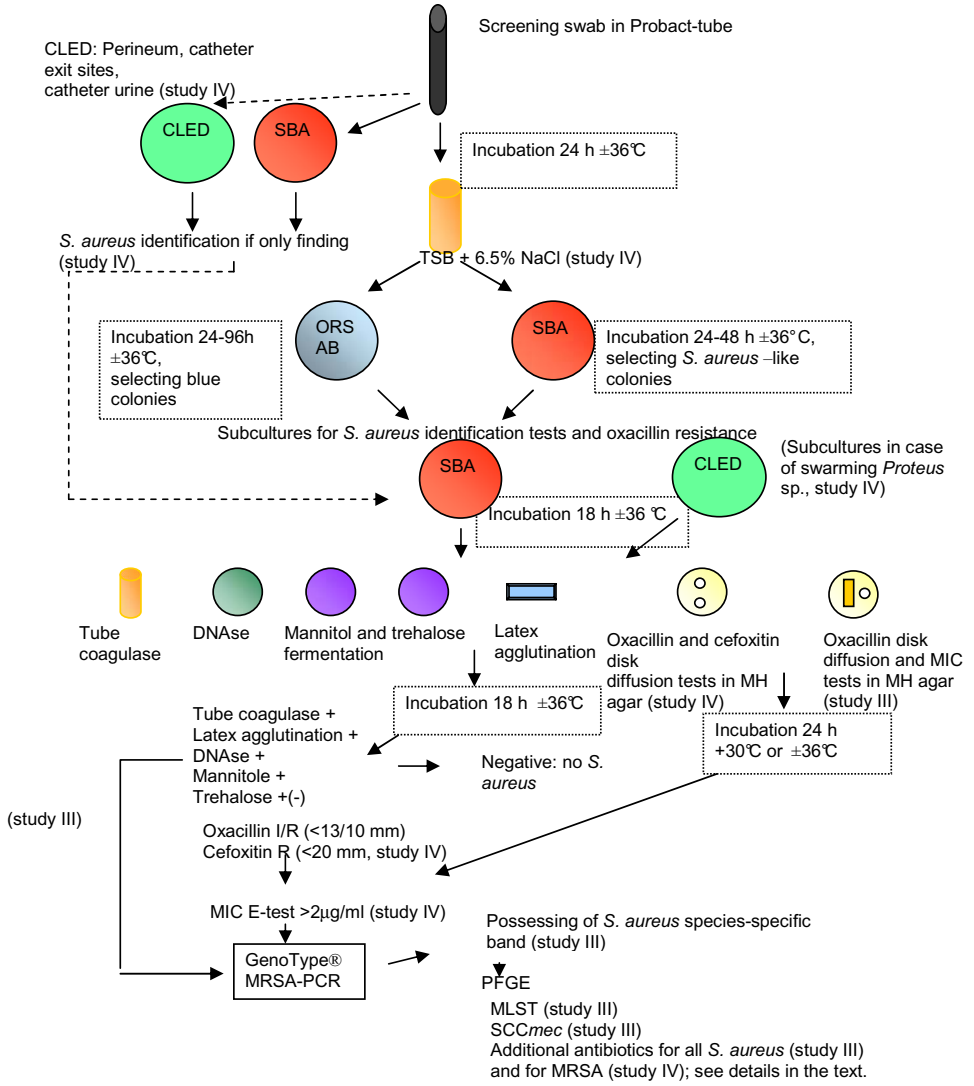

Figure 2. Processing of screening specimens of *S. aureus* and MRSA in studies III and IV. CLED = cystine lactose electrolyte-deficient agar, SBA = sheep blood agar, TSB = trypticase soy broth, ORSAB = Oxacillin Resistance Screening Agar Base, MH = Müller–Hinton agar, MIC = minimum inhibitory concentration, PFGE = pulsed-field gel electrophoresis, MLST = multilocus sequence typing, SCC*mec* = staphylococcal cassette chromosome *mec*.

4.5 Typing methods

4.5.1 Pulsed-field gel electrophoresis (PFGE; studies II, III, IV, and V)

All *S. aureus* isolates in study III, and all genetically verified MRSA isolates in studies II, IV, and V, were genotyped with PFGE as described elsewhere (Murchan et al., 2003). PFGE patterns were analyzed with BioNumerics (version 1.0 or 2.0, Applied Maths, Belgium) by using the Dice coefficient to analyze the similarity of the banding patterns, and with the unweighted pair group method using arithmetic averages (UPGMA) for cluster analysis.

4.5.2 Multilocus sequence typing (MLST; studies II and III)

MLST of the outbreak index strain, and one representative of each MRSA and MSSA strain with a similar PFGE pattern found in study III, and one representative isolate of each PFGE type in study II, was performed as previously described (Enright et al., 2000). Sequences of the seven house-keeping genes were compared to data in the MLST database (www.mlst.net) and the ST was assigned.

4.5.3 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing (studies II and III)

SCC*mec* types of each representative MRSA strain, and all MRSA findings identified with PFGE were determined in studies II and III, respectively. SCC*mec* types were determined with multiplex PCR as described previously (Oliveira and de Lencastre, 2002). *S. aureus* strains HPV 107 (SCC*mec* IA), 96/32010 (SCC*mec* II), HSJ 216 (SCC*mec* type IIIA), and HDE 288 (SCC*mec* IV), a representative of the Iberian, UK-EMRSA-16, Brazilian, and Pediatric clones, respectively, served as SCC*mec* reference strains. *ccrC*-complex (Ito et al., 2004) and IS431-*mecA* (Katayama et al., 2001) were detected with additional pairs of primes.

4.6 Statistical methods (studies I and II)

The Pearson correlation coefficient (and *p*-value) was calculated for the relationship between the rate of MRSA and the number of screening specimens studied to determine the screening activity of hospital districts (I). For categorical variables, proportions were compared by using the chi-square test with Yates correction or Fisher's exact test, as appropriate (II). *p*-values of <0.05 were considered significant.

4.7 Ethical considerations (studies I-V)

Based on Finnish legislation, the KTL must conduct infectious disease surveillance and research in this research area. Similarly, Finnish clinical microbiology laboratories must report certain infectious disease agents and cases, including all MRSA isolations, to the NIDR. Thus, no ethics committee approval was required for our investigation (studies I, II, III, and V). I and my co-workers were at liberty to collect the samples from the residents in study IV with the approval of the

Ministry of Social Affairs and Health as well as of the data protection authority. In addition, permission for sampling was requested from each individual patient.

5. RESULTS

5.1 The changing epidemiology of MRSA (studies I and II)

To analyze the epidemiology of MRSA in Finland, two studies (I and II) were performed, both of which dealt with MRSA notifications, whether the isolates were invasive or found from other sources, as well as with the distribution of MRSA based on the health care institution. A total of 4026 MRSA notifications were reported to the NIDR (120-1458 per year) from 1997 to 2004. The annual incidence of MRSA notifications was 2.3 cases per 100 000 population in 1997, and 27.9 in 2004 (Figure 3). Of the 4026 MRSA notifications, 71 were obtained from blood and 4 from CSF; 30 of the blood isolates and 3 of the CSF isolates were from the year 2004. The proportion of MRSA among blood isolates of *S. aureus* rose from below 1% to 2.8% in 2004. From 1997 to 2004, the numbers of MRSA notifications were strikingly high in three hospital districts (HDs) and in six others experienced an increasing trend (Figure 4). In two HDs, the average annual incidence rate was more than 15 cases per 100 000 population and in six HDs, it varied between 10 and 15 cases (Figure 5). Of the HDs with a rate of ten or more cases per 100 000, two were located in Central Finland, two in Eastern Finland, and three in Northern Finland. The average annual incidence rates of MRSA notifications were lowest in the western parts of Finland.

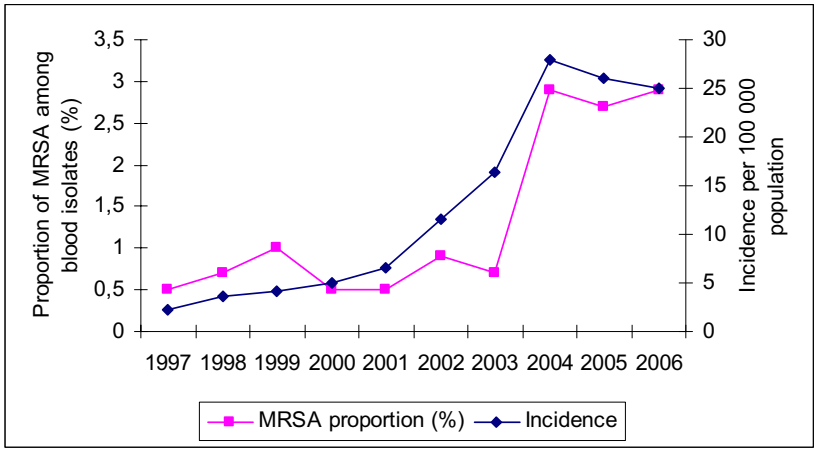

Figure 3. Annual incidence of MRSA notifications and the proportion of MRSA among *S. aureus* blood isolates, Finland, 1997-2006. Source: NIDR

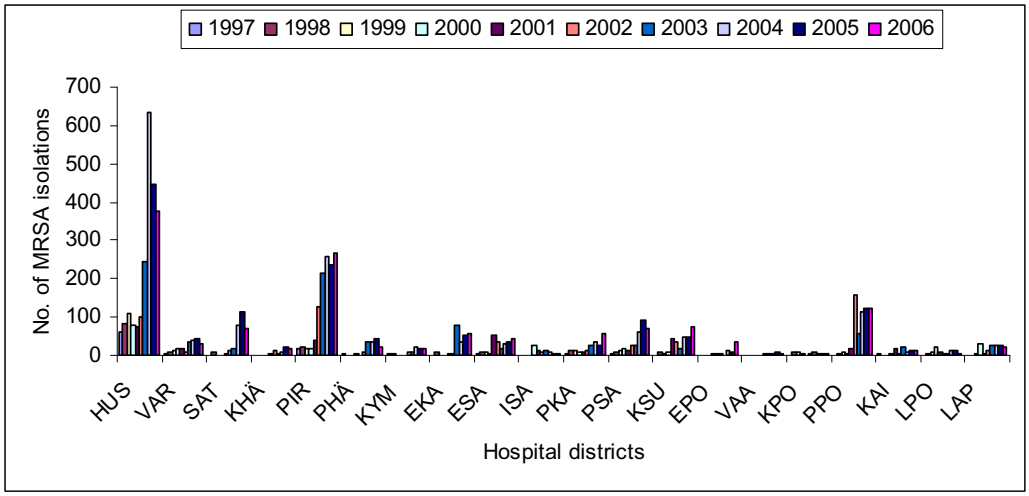

Figure 4. Annual number of MRSA notifications in Finnish hospital districts, 1997-2006. Source: NIDR.

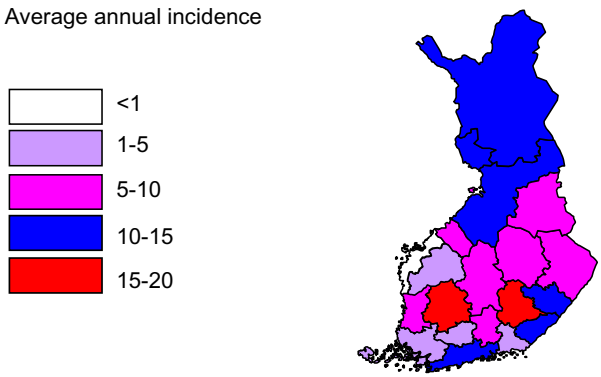

Figure 5. The average annual incidence rate of MRSA notifications per 100 000 population in Finnish hospital districts, 1997-2004.

The age and gender of MRSA-positive persons registered with the NIDR, and the antimicrobial resistance of strains sent to the NRL from 1997 to 2002 were analyzed. The median age of 1718 persons with MRSA was 63 years (range: 0-101) and 49% were male. The proportion of patients aged 65 or more years doubled from 31% to 63% in hospital districts outside the Helsinki metropolitan area. Antimicrobial resistance was studied from a total of 1702 MRSA isolates sent to the NRL. From 1997 to 2002, NMDR isolates of MRSA increased from 41% (56/137) to 67% (387/579), respectively. Of those NMDR isolates, 14% (8/56) and 29% (112/387) were oxacillin-resistant only, respectively. In general, NMDR MRSA was more common outside the Helsinki metropolitan area. Very low-level oxacillin resistance (MIC $\leq$2 µg/ml) was detected in 2% (36/1702) of the MRSA strains. Half of these (18/36) included NMDR, and 10 in 18 of these were resistant only to beta-lactam antibiotics.

Based on the questionnaire, the median number of screening specimens studied was 404 per 100000 population in 2001 (range by hospital district, 0-2768; Figure 6). At the same time, the median rate of MRSA was 11 per 100000 population (range by hospital district, 0-48). The high rate of MRSA failed to correlate with the high number of screening specimens studied (Pearson correlation coefficient 0.18; $p>0.05$). According to the same questionnaire, the CLSI guidelines were variably followed in Finnish microbiological laboratories in 2001. The oxacillin disk diffusion test was carried out in 74% (17/23) of the laboratories, while 35% (8/23) of the laboratories used an incubation period of only 18 h (instead of 24 h) and a temperature of 30°C (instead of 35°C). The amount of salt added to the agar base varied from 0% to 5%, and only 30% (7/23) of the laboratories followed CLSI recommendations, which recommend a 2% NaCl concentration. Ninety-one percent (21/23) of the laboratories reported using the oxacillin-MIC E-test and a specific MRSA screening agar media routinely; 60% of them used ORSAB. A latex agglutination test for the detection of the PBP2a protein was routinely used in 50% of the laboratories. A PCR-based test for *mecA* was used in only one laboratory.

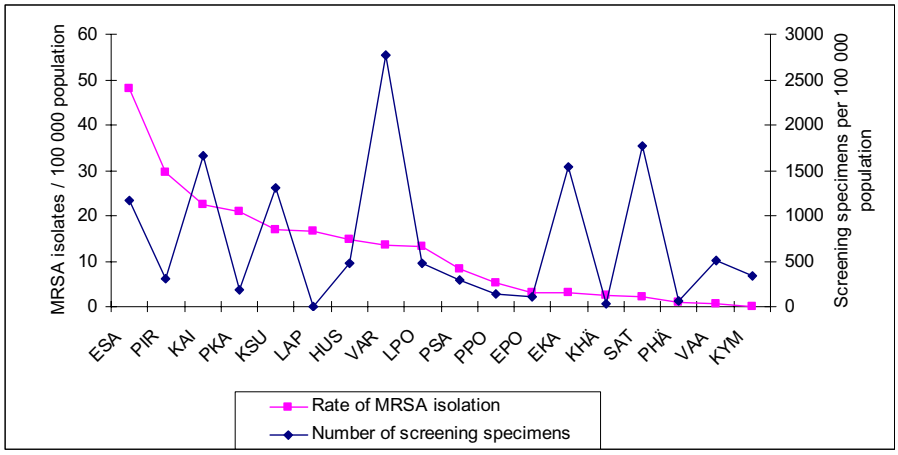

Figure 6. MRSA isolates and the number of screening specimens studied by hospital district in 2001.

Questionnaires about the situation in long-term facilities in 2001 (study I), and about the background information of MRSA-positive persons in 2001-2003 (study II) were sent to the infection control nurses; response rates were 95% (20/21) and 97% (1707/1768), respectively.

MRSA was found in 14 of the 20 HDs in LTFs in 2001. The total number of LTFs involved with MRSA was 58 (39 health care center wards, 11 NHs, 1 center for the mentally disabled, 1 rehabilitation center, and 6 others). Eighty percent (152/189) of the findings were from patients and 20% (37/189) from staff members. The findings comprised more than half of the notifications to the NIDR in 2001 (189/340).

From 2001 to 2003, a total of 1598 (94%) MRSA strains were found among patients and 109 (6%) among staff members. Among the patients, 796 (50%) of the specimens were taken on a clinical basis, 690 (43%) on a screening basis after exposure to MRSA, 65 (3%) on a screening basis because of hospital contact abroad, and 72 (4%) for other reasons. The corresponding figures among the staff members were 12 (11%), 73 (67%), 19 (17%), and 5 (5%), respectively. Screening specimens obtained from patients after exposure to MRSA increased from 36% (96/265) in 2001 to

45% (364/816) in 2003, and those obtained from staff members in epidemic situations increased from 46% (12/26) in 2001 to 82% (36/44) in 2002, but decreased to 64% (25/39) in 2003. A rising trend in the proportion of screening specimens was observed in seven HDs, including two HDs with a strikingly high rate of MRSA notifications. In nine HDs, no such trend occurred and in four HDs, the proportion decreased (Table 5).

Table 5. The numbers and proportions of clinical and screening findings of MRSA by hospital districts (HD) during the study period 2001-2003 in Finland

HD	no. of clinical finding			no. of screening findings			clinical-%			screening-%		
	2001	2002	2003	2001	2002	2003	2001	2002	2003	2001	2002	2003
HUS	31	44	110	15	35	116	67	56	49	33	44	51
VAR	4	3	8	10	5	27	29	37	23	71	63	77
SAT	1	5	7	3	6	9	25	45	44	75	55	56
KHÄ	3	4	2	1	10	0	75	29	100	25	71	0
PIR	36	80	113	5	46	124	88	63	48	12	37	52
PHÄ	2	7	17	0	2	14	100	78	55	0	22	45
KYM	0	1	1	0	4	8	0	20	11	0	80	89
EKA	3	2	9	2	0	70	60	100	11	40	0	89
ESA	17	15	13	46	20	3	27	43	81	73	57	19
ISA	8	8	8	6	2	0	57	80	100	43	20	0
PKA	7	10	12	5	9	13	58	53	48	42	47	52
PSA	13	13	14	6	10	10	68	57	58	32	43	42
KSU	9	16	13	21	12	1	30	57	93	70	43	7
EPO	3	5	2	2	0	0	60	100	100	40	0	0
VAA	0	3	3	0	0	3	0	100	50	0	0	50
KPO	1	3	7	0	1	2	100	75	88	0	25	12
PP0	14	22	22	5	129	32	67	14	41	33	86	59
KAI	9	2	6	10	1	14	47	67	30	53	33	70
LPO	1	3	3	5	1	2	17	75	60	83	25	40
LAP	4	6	4	4	8	20	50	43	17	50	57	83

5.2 Nationwide trends in the molecular epidemiology of MRSA (study II)

To assess recent trends in the molecular epidemiology of Finnish MRSA strains, a total of 4091 MRSA isolates sent to the NRL from 1997 to 2004 were analyzed. With PFGE, 253 different strain types of which 38 were outbreak/epidemic and 215 sporadic strains were identified. From 1997 to 2004, 24 new outbreak/epidemic strain types appeared (Table 6). The proportion of sporadic strains varied between 3% and 13% during the study period, but overall showed a decreasing trend.

Table 6. Thirty-eight outbreak/epidemic MRSA strain types appeared in Finland during the study period 1997-2004. Five strain types in parentheses () have not been found since 1997, and were not included in the original version of study II. Antimicrobials in the squared brackets [ ] indicate variable resistance between the isolates. Cip=ciprofloxacin, Gen=gentamicin, Tob=tobramycin, Ery=erythromycin, Clin=clindamycin, Chlor=chloramphenicol, Rif=rifampin, Fus=fusidic acid, Tri+Su=trimethoprim-sulfamethoxazole, Tet=tetracycline, and Mup=mupirocin. HD means hospital district.

FIN-name	**Old name**	**Year of first isolation in Finland**	**No. of HD in which the strain was found, 1997-2004 (total n=20)**	**PFGE-band difference / % difference**	**MLST**	**SCC*mec***	**PVL**	**Antimicrobial resistance other than to betalactams**
FIN-1	Turku I	1991	4		247	IVA	-	Gen, Tob, Ery, Tet, Cip, Clin, Rif
FIN-1a	Turku II	1992	1	3/90,32	247	IA	-	Gen, Tob, Ery, Tet, Cip, Clin, Rif
FIN-1b	Turku III	1992	2	4/87,5	247	IVA	-	Gen, Tob, Ery, Tet, Cip, Clin
(FIN-1c)	HKI II	1993	ND	6/81,25	247	IVA	-	Gen, Tob, Ery, Tet, Cip, Clin, Chlor, Rif
(FIN-1d)	Kotka	1993	ND	4/86,67	247	IVA	-	Gen, Tob, Ery, Tet, Cip, Clin
FIN-1e	Tampere III	1994	3	6/81.25	572	IA	-	Gen, Tob, Ery, Tet, Cip, Clin, Rif
(FIN-2)	Seinäjoki	1992	ND		239	IIIA	-	Ery, Tet, Clin, Chlor, Tri+Su
FIN-2a	Turku IV	1993	2	6/ 80.0	239	IIIA	-	Ery, Tet, Tri+Su
(FIN-2b)	HKI IV	1994	ND	6/81,25	239	III	-	Gen, Ery, Tet, Clin, Chlor
FIN-2c	Liettua	2000	2	6/81,25	239	III	-	Gen, Tob, Ery, Tet, Cip, Chlor, Fus
FIN-2d	HKI VI	1998	2	6/77,42	239	III	-	Gen, Ery, Tet, Cip, Clin, Tri+Su
FIN-2e	Lohja	1998	7	6/78,57	241	IIIB	-	Gen, Tob, Ery, Tet, Cip, Clin, Chlor, Tri+Su
FIN-3	HKI I	1992	14		5	II	-	Gen, Tob, Ery, Cip, Clin
FIN-3a	Koskela	1999	1	6/86,67	5	II	-	Tob, Ery, Cip, Clin
FIN-4	Mikkeli II	1997	18		375	IV	-	-
FIN-4 a	Mikkeli I	1993	13	2/92,86	375	IV	-	-
FIN-4 b	Tampere I	1994	2	1/96,3	375	IV	-	-
FIN-5	Pori I	1993	11		30	IV	-/+	Gen, Tob, [Ery]
FIN-5a	HKI V	1995	11	5/80	36	II	-	Gen, Tob, Ery, Cip, Clin, Mup
FIN-7	Kokkola	1997	16		8	IV	-	-
FIN-7 a	Pori II	1993	1	0/100	8	IV	-	-
(FIN-8)	HKI III	1994	ND		5	IVA	-	Gen, Tob, Ery, Cip, Clin, Rif
FIN-9	Tampere II	1994	1		239	IIIA	-	Gen, Tob, Ery, Tet, Cip, Clin, Chlor, Rif

Table 6. (*continued*)

FIN-name	Old name	Year of first isolation in Finland	No. of HD in which the strain was found, 1997-2004 (total n=20)	PFGE-band difference / % difference	MLST	SCC*mec*	PVL	Antimicrobial resistance other than to betalactams
FIN-10	Kemi	1996	16		45	IV	-	Ery [Cip]
FIN-10 a	Joensuu I	1996	1	1/ 96.0	45	IV	-	-
FIN-10 b	Kajaani	1997	6	1/96.0	45	IV	-	-
FIN-10 c	Berlin IV	1998	6	5/81.48	45	IV	-	-
FIN-10 d	Pello	2000	12	2/92.31	45	V	-	- [Gen, Tob, Tet, Tri+Su]
FIN-11	HKI VIII	1997	17		80	IV	+	- [Ery, Tet, Clin, Cip]
FIN-12	UK EMRSA-15	1997	16		22	IV	-	Ery, Cip
FIN-13	HKI VIIb	1998	2		239	III	-	Gen, Tob, Ery, Tet, Cip, Clin
FIN-14	Joensuu II	1998	7		12	IV	-	-
FIN-15	Kerimäki	2000	7		8	IV	-	Ery
FIN-16	Bel EC-3	2001	15		125	IA	-	Tob, Ery, Cip, Clin
FIN-17	Karkkila	2000	2		46	V	-	Cip
FIN-18	Moskova	2000	6		8	IV	-	Gen, Tob, Ery, Cip, Clin, Chlor
FIN-19	Nurmes	2001	6		1	IV	-/+	-
FIN-20	Vaalijala	2001	6		72	IVA	-	Tob
FIN-21	Töölö	2002	3		228	I	-	Gen, Tob, Ery, Cip, Clin
FIN-22	No name	2003	2		27	V	-	-
FIN-23	No name	2004	1		228	IV	-	Gen, Tob, Ery, Cip, Clin, Tri+Su
FIN-24	No name	2004	1		111	IV	-	Gen, Tob, Ery, Cip, Clin, Tri+Su
FIN-25	No name	2004	1		8	IV	+	Ery

At the beginning of the study period (1997-2001), the distribution of different strain types was more diverse, but since 2002, five strain types were the most predominant (FIN-16 (ST125:IA), FIN-4 (ST375:IV), FIN-21 (ST228:I), FIN-7 (ST8:IV), and FIN-10 (ST45:IV and V) (Figure 7). In 2004, more than half of the strains (53%) found were comprised of FIN-16 and FIN-21 (ST228:I) strains. FIN-16 increased from <1% in 1997 to 25% in 2004 and was found in 15 HDs (Table 6), but the majority (554/742; 75%) of all FIN-16 strains was found in one HD in Central Finland. The proportion of the FIN-21 strain increased from 6% in 2002 to 28% in 2004, and only a minority of the strains were found outside the Helsinki metropolitan area (9/581; 2%). The most common MRSA isolates found from blood and from CSF in 2004 were FIN-16 (12/34), FIN-21 (9/34), and FIN-4 (4/34); no significant associations were found either between the type of specimen (blood and CSF vs. other) and these strains in 2004 or during the entire study period.

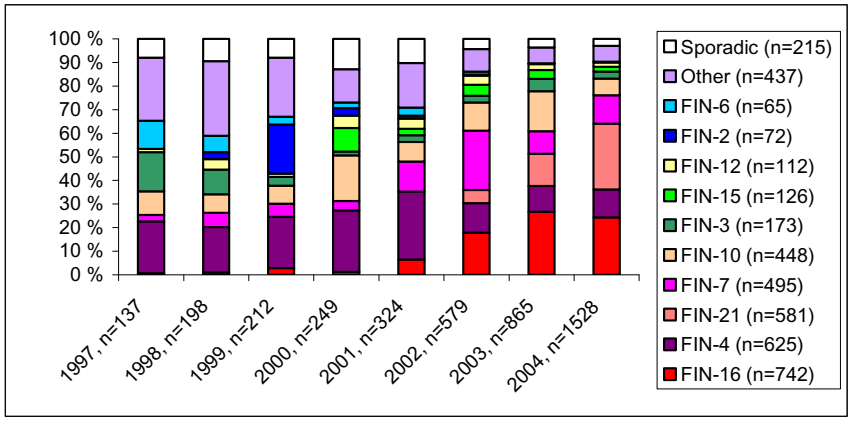

Figure 7. The distribution of MRSA strains, Finland, 1997-2004.

FIN-4 (including also FIN-4a and FIN-4b strain types; ST375:IV) was the second most often isolated strain, and was found in every HD (range annually 7-17 HD) in Finland during the study period (Table 6). FIN-7 (ST8:IV) and FIN-10 (ST45:IV or V) strains have also been found in the majority of HDs, but mostly in three (341/495; 69%) and six (336/448; 75%) HDs, respectively (the annual range in the number of hospital districts: 3-13 and 3-15, respectively).

Combining the additional background information on MRSA-positive persons (see also results in 5.1), with the PFGE results showed that FIN-16 strains were more likely to occur in clinical specimens than in those obtained on a screening or other basis (199 [25%] of 805 vs. 160 [18%] of 902; $p < 0.05$) (Table 7). On the contrary, FIN-21 strain was tended to be found more often from screening and other specimens than from those taken on clinical basis (58 [7%] of 805 vs. 87 [10%] of 902; $p=0.086$). FIN-4 was more likely to occur among isolates obtained from patients and staff members on a clinical basis than in screening (167 [21%] of 805 vs. 81 [9%] of 902; $p<0.05$). FIN-7 (ST8:IV) and FIN-10 (ST45:IV) were more likely to occur in screening specimens of patients and staff members than in their clinical specimens (70 [9%] of 805 vs. 194 [22%] of 902; $p<0.05$, and 89 [11%] of 805 vs. 149 [17%] of 902 ; $p<0.05$).

Of the representative strains, FIN-11 (ST80:IV) and FIN-25 (ST:IV) tested PVL positive (Table 6). In addition, some strains of FIN-5 (ST-30:IV) and FIN-19 (ST1-IV) possessed *lukS*-PV - *lukF*-PV genes that encode for PVL (Table 6).

Table 7. Background information on patient and staff members who tested positive for the ten most common epidemic MRSA strains, 2001-2003.

MRSA strain	Number of patients (1598)					Number of staff members (109)				Total no. of isolates
	Sequence type (ST) and SCC*mec* type	Clinical specimen	Screening specimen; exposure to MRSA	Screening specimen; hospital contact abroad	Other reason for taking the sample	Clinical specimens	Screening specimen; epidemic situation	Screening specimen; hospital contact abroad	Other reason for taking the sample	
FIN-16	ST125:IA	197	131	5	18	2	3	1	2	359
FIN-7	ST8:IV	70	159	1	6	-	28	-	-	264
FIN-4	ST375:IV	163	56	5	12	4	8	-	-	248
FIN-10	ST45:IV, V	87	129	2	6	2	12	-	-	238
FIN-21	ST228:I	58	82	1	3	-	1	-	-	145
FIN-3	ST5:II	22	30	8	7	-	4	-	-	71
FIN-15	ST8:IV	34	19	-	5	-	9	-	1	68
FIN-12	ST22:IV	19	12	8	4	-	1	14	-	58
FIN-6	ST36:II	5	1	5	-	-	1	1	-	13
[a]FIN-2	ST241:IIIB	7	-	2	-	-	-	-	-	9
Other strains		79	49	8	2	3	5	2	2	150
Sporadic		52	22	19	2	1	1	1	-	98
Total		793	690	64	65	12	73	19	5	[b]1721

[a]Of the FIN-2 strain, the FIN-2e subtype caused the epidemic.
[b]The total number of strains (1721) exeeds the number of MRSA-positive persons (1707) by 14 because in some cases more than one reason for sampling was chosen in the questionnaire.

5.3 Methicillin-sensitive *S. aureus* (MSSA) and MRSA in Finnish long-term facilities (studies III and IV)

5.3.1 Prevalence of MSSA and MRSA among long-term facility residents

At the time of the point-prevalence survey carried out in Northern Finland (study III), a total of 90 specimens, 76 from the nostrils, 10 from skin lesions, and 4 from catheter urine, were obtained from 76 of 80 in-patient volunteers (Table 8). Of the 90 specimens, 30 (33%) tested positive for *S. aureus* and 5 (17%) tested positive for MRSA. Of the 76 patients, 24 (32%) were *S. aureus* carriers and 5 (20%) tested positive for MRSA; the prevalence of MRSA was 7%. Two of the five MRSA isolates came from patients who tested positive during the outbreak in 2003 (one from a NH, and another from a HCW), one of the patients tested positive for MRSA for the first time nine days before the point-prevalence survey in February 2004 (from a NH), and two of the patients were new carriers (both from a NH). Of the 13 patients who previously tested positive for MRSA during the outbreak in 2003, five died, one was discharged, and five were culture-negative for MRSA at the time of the point-prevalence survey.

In study IV, different body sites were evaluated for their ability to detect *S. aureus* carriage. Of the 217 residents present at the selected ward in the largest NH in the Helsinki metropolitan area, 4 completely refused sampling, and of the remaining 213, 3 (1.4%) refused nostril swabbing, 15 (7.0%) throat swabbing, and 8 (3.8%) perineal swabbing. A total of 663 specimens (median: 3; range: 1-7) were obtained from 213 patients: 165 specimens (25%) from 94 patients (44%) tested positive for *S. aureus,* and 3 specimens (0.4%) from 2 (0.9%) patients in the same ward tested positive for MRSA (Table 8). The highest proportion of *S. aureus* growth was obtained from the catheter exit sites (56%, 5/9), and lowest from the perineum (13%, 27/207) and catheter urine (19%, 3/16). Of the 165 *S. aureus* isolates, 32 (19%) were obtained only from sites other than the nostrils from a total of 25 patients: 11 specimens came from the throat, 7 from the perineum (6 MSSA and 1 MRSA), 2 from skin lesions, 1 from the catheter exit site, 6 from a combination of the throat and perineum of three patients, 2 from a combination of the perineum and a skin lesion of one patient, and 3 from a combination of the throat, perineum, and a skin lesion of one patient.

Table 8. The prevalence of *S. aureus* (MSSA and MRSA) isolates among 90 and 663 specimens from 76 and 213 residents in a Finnish nursing home from studies III and IV, respectively.

Screening site	**Total no. of specimens, studies III / IV**	**No. of specimens, positive for *S. aureus* (%), studies III / IV**	**Growth of *S. aureus* only with TSB enrichment (%), study III**	**No. of specimens, positive for MRSA, studies III / IV**	**Growth of MRSA in ORSAB, studies III / IV**
Nostrils	76 / 210	24 (32) / 69 (33)	10 (14)	4 / 1	2 / 1
Throat	ND / 198	ND / 53 (27)	7 (13)	ND / -	ND / -
Perineum	ND / 207	ND / 27 (13)	13 (48)	ND / 1	ND / -
Skin lesion	10 / 23[a)]	5 (50) / 8[b)] (35)	3 (38)	1 / -	1 / -
Catheter exit site	ND / 9	ND / 5 (56)	-	ND / 1	ND / 1
Catheter urine	4 / 16	1 (25) / 3 (19)	-	- / -	-/-
Total	90 / 663	30 (33) / 165 (25)	33 (20)	5 / 3	3 / 2

[a)] Taken from 16 residents.
[b)] Found in seven residents.

Table 9. The genotypes and antimicrobial resistance of MRSA and MSSA strains determined with PFGE in studies III and IV. A minus (-) means not found.

PFGE strain name	No. of isolates		No. of patients		Antimicrobial resistance of MRSA / MSSA
	MRSA, studies III / IV	MSSA, study III	MRSA, studies III / IV	MSSA study III	
FIN-22	2 / -	3	2 / -	3	beta-lactams / none
FIN-21	- / 2	-	- / 1	-	beta-lactams gentamycin tobramycin erytromycin clindamycin ciprofloxacin
FIN-7	3 / 1	3	3 / 1	3	beta-lactams / none
FIN-14	- / -	5	- / -	3	- / none
FIN-10	- / -	6	- / -	6	- / none
FIN-16	- / -	1	- / -	1	- / none
Sporadic	- / -	7[a]	- / -	6	- / none[b]
Total[c]	5 / 3	25	5 / 2	22	

[a] One patient in study III had two identical sporadic MSSA strains in different sites.
[b] One of the seven sporadic strains was resistant to chloramphenicol.
[c] The total number of patients (27) exceeds the real number (24) by three because of duplicate genotype findings in study III.

5.3.2 Detection of MSSA and MRSA among long-term facility specimens

Screening specimens of *S. aureus* and MRSA obtained from two Finnish LTFs were investigated at the NRL. All five MRSA isolates found in study III were resistant only to oxacillin (Table 9), expressing an oxacillin MIC of 2-32 mg/l. Two isolates found in new carriers expressed oxacillin MICs of 2 and 4-6 mg/l, respectively, failed to grow on the ORSAB with 2 mg/l of oxacillin. All 25 MSSA strains were sensitive to all antimicrobials tested, except one strain, which was resistant to chloramphenicol (Table 9).

In study IV, the necessity of performing broth enrichment to increase sensitivity, and the usability of ORSAB, which is widely used in routine diagnostics in Finland were assessed. TSB enrichment detected an additional 33 (5%) *S. aureus* isolates (32 MSSA, and 1 MRSA) in a total of 28 patients compared to both selective and non-selective agar plating, which resulted in finding 8 (4%) additional *S. aureus*-positive patients (Table 8). Four *S. aureus* isolates (0.6%), two from the nostrils and two from the throat, were found only in SBA used as a control plate for TSB in a primary culture. Four *S. aureus* isolates were isolated only in CLED, of which two were from primary cultures of the perineum, and two from subcultures of the nostrils because of swarming *Proteus* sp. Within 96 h, 187 of the 663 (28%) specimens grew in ORSAB, 2 (1%) of which were MRSA. Of the 187 cultures, half (94/187) were from the perineum, and 46 (25%) developed blue colonies within 24 h. The two multi-drug-resistant MRSA strains isolated from the nostrils and a catheter exit site in the same resident grew in ORSAB within 24 h, but the non-multi-drug-resistant MRSA strain, isolated from the perineum and expressing an MIC of 6 mg/l, did not.

5.3.3 Molecular epidemiology of MSSA and MRSA in long-term facilities

In study III, a total of 30 *S. aureus* isolates obtained from 24 patients were analyzed with PFGE, and 11 different PFGE profiles were detected (Table 9). Of five MRSA isolates, two isolates showed a FIN-22 PFGE profile and three isolates showed a genotype of another Finnish epidemic, the FIN-7 PFGE profile MRSA strain. MLST characterized two representative strains of FIN-22 and FIN-7 (one MRSA and one MSSA) as ST-27 and ST-8, respectively. All five MRSA strains possessed the F-locus typical for SCC*mec* type III (414 bp) detected by multiplex-PCR. The strains tested negative, however, for recombinase genes *ccr*A and *ccr*B, but possessed *ccr*C detected with specific primers; thus the strains belonged to SCC*mec* type V (Figure 8). None of the 30 *S. aureus* (MRSA and MSSA) strains tested positive for the PVL gene.

Three of the MSSA strains were genotypically related to the FIN-22 strain, and three to the FIN-7 MRSA strain (Table 9). Twelve MSSA strains from ten different patients were related to three other Finnish epidemic MRSA strains, and seven strains from six different patients were considered sporadic. One patient was colonized with two genotypically different MSSA strains: FIN-7 was found in his nostril and FIN-10 in his skin lesion. Two patients were widely colonized with the same MSSA strain: one (FIN-14) was found in a nostril, urine, and skin lesion, and another (sporadic) strain in a nostril and skin lesion. The two patients colonized with both MRSA and MSSA possessed strains of different genotypes. One had the FIN-22 MRSA strain in his skin lesion located on his back, and the FIN-10 MSSA was found in his nostril; the other patient had both FIN-7 MRSA and FIN-10 MSSA in her nostril.

In study IV, two MRSA strain types were identified with PFGE typing: non-multidrug-resistant strain FIN-7 in one patient, and a multidrug-resistant strain FIN-21 in another patient (Table 9).

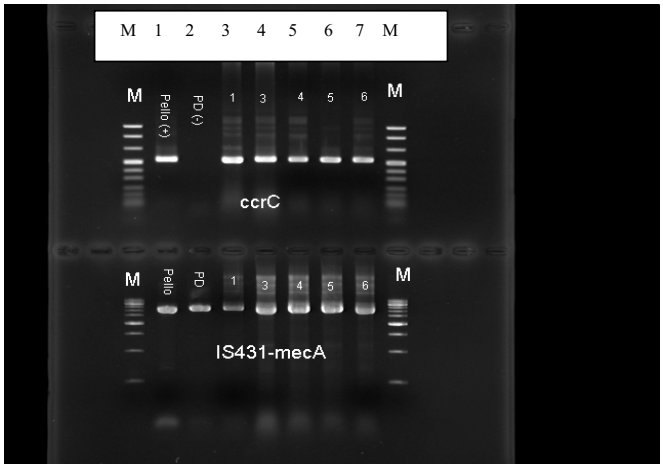

Figure 8. Patient MRSA strains 1-5 (lanes 3-7), positive control for SCC*mec* type V (FIN-10; lane 1), negative control for SCC*mec* type V (Pediatric; lane 2), and the DNA-size molecular marker pUC (in upper gel; lane M), and XVII (in lower gel; lane M).

5.4 Phenotypic detection of MRSA (study V)

With cefoxitin disk diffusion using interpretive zone diameters of R ≤ 19 mm, 100% sensitivity and 100% specificity at +35°C with or without the addition of salt according to *mecA* status was obtained (Table 1). When MH plates were incubated at +30°C, the specificity remained at 100%, but sensitivity decreased to 94.7% and 97.3% with or without the addition of salt, respectively. With the oxacillin disk diffusion test, a sensitivity of 100%, but 99.3% when incubating at +30°C without the salt addition was obtained. Specificity was 35.7% and 50% at +35°C with or without the salt addition, respectively, and 21.4% and 42.9% at +30°C with or without the salt addition, respectively. With the oxacillin E-test, a sensitivity of 94% at both +35°C and +30°C without the salt addition and 99.3% at +35°C and 98.7% at +30°C, both with the salt addition was obtained. Specificity was 57.1% at both +35°C and +30°C with the salt addition, and 92.8% at +35°C and 78.6% at +30°C, both without the salt addition.

Table 10. Comparison of resistance determination performed by oxacillin and cefoxitin disk diffusion methods and E-test MIC-method in *mecA*-positive and -negative *S. aureus* strains.

Number of strains								
	Incubated with MH in +35°C		**Incubated in MH+NaCl +35°C**		**Incubated with MH in +30°C**		**Incubated in MH+NaCl +30°C**	
Oxacillin 1 µg								
	≤12 mm	≥13 mm	≤12 mm	≥13 mm	≤12 mm	≥13 mm	≤12 mm	≥13 mm
Positive (150)	150	0	150	0	149	1	150	0
Negative (14)	7	7	9	5	8	6	11	3
Cefoxitin 30 µg								
	≤19 mm	≥ 20 mm	≤19 mm	≥ 20 mm	≤19 mm	≥ 20 mm	≤19 mm	≥ 20 mm
Positive (150)	150	0	150	0	146	4	142	8
Negative (14)	0	14	0	14	0	14	0	14
Oxacillin-MIC								
	≥3	≤2	≥3	≤2	≥3	≤2	≥3	≤2
Positive (150)	141	9	149	1	141	9	148	2
Negative (14)	1	13	6	8	3	11	6	8

5.5 Genotypic verification of MRSA (study V)

All 164 *S. aureus* isolates tested positive for the *nuc* gene, and 150 of those also tested positive for the *mecA* gene with the used in-house PCR tests. With Geno Type® MRSA, both the detection of *mecA* and amplicons specific to the *S. aureus* species corresponded perfectly with the results obtained with in-house PCR. Geno Type® MRSA also correctly detected all 42 *Staphylococcus epidermidis* strains, of which 38 possessed the *mecA* gene. The results for both *Enterococcus* isolates and all ATCC reference strains were in concordance with those of in-house PCR. The EVIGENE™ MRSA Detection test correctly identified all 164 *S. aureus* strains that tested positive for *nuc*, and 150 strains that tested positive for *mec*A. With three *S. aureus* strains, the *nuc* gene was slightly below the cut-off level, but the strains were visually interpreted as positive.

6. DISCUSSION

6.1 The changing epidemiology and molecular epidemiology of MRSA in Finland

MRSA has become a persistent problem worldwide. Rising MRSA rates have also been observed in Finland, especially among the elderly. The present study shows a consistent year-to-year rise in the annual number of MRSA isolates in Finland from 1997 to 2004. Before the year 2003, the rise occurred mostly outside the Helsinki metropolitan area where NMDR MRSA also was more common. In the Helsinki metropolitan area, MDR MRSA dominated the entire study period. MDR MRSA is typically found more often in acute care hospitals, and NMDR outside hospitals (Salmenlinna et al., 2002). The rise in the incidence of MRSA, compared to that of the entire country, remained one of the lowest in Southwestern Finland. The screening activity was, however, the highest in Southwestern Finland during 2001. In general, the screening activity failed to correlate with the rate of MRSA. HDs with a low rate of MRSA were usually characterized by high screening activity and *vice versa*. This could be due to a different screening activity policy between HDs or to an ongoing outbreak in that year.

In 2001, the number of MRSA cases rose among elderly persons outside the Helsinki metropolitan area, and 58% of all persons with MRSA were found in nursing homes. The survey on the MRSA situation, however, covered only one year, and the clinical data and risk factors for MRSA acquisition in Finnish long-term facilities were unavailable. It can only be assumed that most of the MRSA findings in Finnish long-term care facilities were found from screening specimens, since it is generally known that clinical infections are rarer in long-term care than in acute-care hospitals (Bradley, 1999).

The most recent rise was related to the spread of two internationally recognized MDR MRSA strains, FIN-16 (ST125:IA) and FIN-21 (ST228:I), which also caused a striking increase in blood and CSF findings. A similar rise in ST125:IV clones in Spanish hospitals was also reported (Perez-Roth et al., 2004). A single ST125 strain has also been found in Norway (http://www.mlst.net), but the SCC*mec* type was not reported. Most of the FIN-16 strains were found from clinical specimens, and FIN-16 was the most common strain isolated from invasive specimens in 2004, indicating that it may be virulent and have epidemic potential. Since FIN-16 dominated one HD for four years, it can be considered as an endemic strain in that area, especially in long-term facilities. Thus, it can equally be assumed that its capacity to cause clinical infections occurs simply because the FIN-16 strain has reached a sufficiently high level among the population, especially among the oldest and diseased patients who are transferred between LTFs and acute-care hospitals. The FIN-21 strain was found in Finland for the first time in September 2002 (Kanerva et al., 2007), and only few of these strains were found outside the Helsinki metropolitan area. In the NH in the Helsinki metropolitan area, two of the three MRSA strains found there were FIN-21, and only one patient was colonized with this strain. This was quite an unexpected result, since this strain has recently caused many epidemics in secondary- and tertiary-care hospitals in the Helsinki metropolitan area. The strain had a PFGE pattern similar to the Italian clone (Mato et al., 2004), and expressed also MLST allelic profile ST 228, and carried SCC*mec* type I. Isolates of MRSA with the same ST228 were reported as a “South Germany” epidemic clone (Cookson et al., 2007; Murchan et al., 2003; Petersdorf et al., 2006). The strain was isolated more often from screening specimens than from those taken on a clinical basis, contrary to the tendency with FIN-16. This may be due to differences in MRSA screening activity between the HDs, or to the subsequent appearance of the strain in Finland. The

outbreaks differed, however, in their epidemiology: FIN-16 prevailed in LTF and FIN-21 in acute-care hospitals.

Almost 60% of the MRSA strain types carried SCC*mec* IV or V, which are often seen in CA-MRSA (Ito et al., 2004; Zetola et al., 2005). However, only four strain types harbored PVL encoding genes, another marker that has been used to identify of CA-MRSA (Miller et al., 2007; Seybold et al., 2006) and has been linked to the development of furuncles, abscesses, and severe necrotic infections (Lina et al., 1999). The majority of all Finnish MRSA strains previously associated with community acquisition possessed type IV SCC*mec*, but only a minority (12%) of them were PVL positive (Karden-Lilja et al., 2007). As reported elsewhere, most of the PVL-positive strains, however, were ST80 (Faria et al., 2005; Witte et al., 2005). FIN-4 (ST375), the most commonly found strain possessing SCC*mec* type IV, was associated with clinical infections among patients more often than the two other commonly found SCC*mec* IV-expressed strains, FIN-7 (ST8) and FIN-10 (ST45). A previous Finnish study covering the years 1997-1999 showed that the FIN-4 strain was one of the three epidemic strains found most often in patients with no health care setting contact, thus suggesting that these MRSA strains were community acquired (Salmenlinna et al., 2002). Unfortunately, no health care records were available in this present study, so it cannot be assessed whether acquisition of these three strains is still associated with the community. Interestingly, FIN-4 strains, which were not considered internationally spread epidemic strains, were found in five patients with hospital contact abroad. Although those strains may also be endemic elsewhere in the world, these patients may have been infected with MRSA in Finland before or after their hospital contact abroad. However, ST375 has recently been shown to spread in Denmark, where it has been identified as CA-MRSA (Faria et al., 2005).

The FIN-7 strain was the fourth most common strain type found in our study. It belongs to ST8 clones, which occur worldwide, and have been reported to express four different SCC*mec* types (I-IV), mainly type IV (Aires de Sousa and de Lencastre, 2003; Chung et al., 2004; Enright et al., 2002). ST8:IV strains have been reportedly associated with community acquisition (Carleton et al., 2004), although they have recently been reported to cause infections in health care facilities (Seybold et al., 2006). FIN-7 was also found in residents who tested positive for MRSA in both NH studies, although these strains expressed type V SCC*mec*.

The fifth most commonly found strain type was FIN-10, which belongs to the ST45 clones. ST45 clones have been reported to spread widely both as MSSA and as MRSA in Scandinavia, Western Europe, and the USA (Enright et al., 2002). Moreover, ST45 clones were also found in a hospital in Miami, Florida, where they were considered community-acquired (Chung et al., 2004). Wannet and colleagues have reported that MRSA ST45 was recognized for the first time in the Netherlands in 2000 and reached epidemic proportions in 2002 (Wannet et al., 2004). In contrast to other studies, they described MRSA ST45 as having low-level resistance (4 to 32 mg/liter; median: 8 mg/liter) to oxacillin. The Finnish main representative strains of ST45:IV and ST8:IV (FIN-10 and FIN-7, respectively) were resistant to oxacillin alone and expressed a heterogeneously resistant phenotype.

The annual proportion of sporadic strains has decreased during the study period, although their total number has remained almost unchanged or has slightly increased, indicating that new strain types still emerge. As the routine typing repertoire and the MRSA PFGE database have increased in volume, it is likely that common ancestors can more easily be found for a greater number of "sporadic" strains. On the other hand, sporadic strains have been speculated to possess a worse environmental survival capacity than epidemic strains (Wagenvoort et al., 2000). Sporadic strains are thought to be more often community acquired, whereas hospital-acquired MRSA strains are more often endemic (Blanc et al., 2002; Petersdorf et al., 2006). Most of Finnish sporadic strains

were found in the clinical specimens, but one fifth of them originated from persons who had had hospital contact abroad. Blanc and colleagues have reported that at least one-third of the sporadic strains found in Switzerland resulted from the ongoing introduction of new strains from abroad (Blanc et al., 2002). They have also shown that most Swiss sporadic isolates were related to other European epidemic MRSA clones (Blanc et al., 2000). One cannot be sure of the origin of Finnish sporadic strains because the routine molecular typing scheme is based primarily on the highly discriminatory PFGE, which allows the detection of genomic microevolution, unlike MLST. Finnish sporadic strains may have descended from a few ancestors, a fact which could have been detected with MLST and eBURST as well as with spa typing.

Because the NIDR notifications include only the source of the specimen, rather than whether the specimen is taken on a screening or clinical basis or whether further data on clinical specimens/infections are available, the survey was performed in 2001-2003 in order to make this crucial distinction. The proportion of screening specimens among the patients and staff members increased during the study period, and almost half of the MRSA findings were found in patients' screening specimens. This active screening policy may partly be related to the rise in the number of MRSA cases that was detected, but likely had no influence on the worrying trend in blood and CSF findings. Thus, it is believed that the spread of MRSA has increased, as indicated by each new MRSA case. It is also very likely that there are some differences not only in resources for, but also in experiences with MRSA control in long-term care as well as in health care settings.

6.2 *S. aureus* in Finnish long-term facilities

6.2.1 MSSA and MRSA colonization among Finnish long-term facility residents

In the Northern Finnish LTFs, the point-prevalence survey showed that the prevalence of MRSA was 7%, and that of MSSA, 32%. In the second largest Finnish NH in the Helsinki metropolitan area, MSSA colonization was 44%, but the prevalence of MRSA was low: 0.9%. The MRSA colonization rates differed between these facilities due to an outbreak of MRSA in the Northern LTF. Differences in colonization rates may also depend on the presence of invasive devices and skin lesions, the type and severity of the underlying conditions of residents, and infection control practices at the LTF (Bradley, 1999). One limitation of the study III was that only nostrils of all the participating patients were screened, although skin lesions were also screened if present. Studies have reported that a combination of three body sites (nose, throat, and wounds/skin lesions) are necessary to detect all MRSA (Muto et al., 2003; O'Sullivan and Keane, 2000b). However, with the methods that were used, it was possible to detect two new carriers who tested MRSA-negative nine days before being screened by local authorities.

In study IV, different body sites for the ability to detect *S. aureus* carriage were evaluated. The highest rates of *S. aureus* colonization in catheter exit sites were detected, although only a few residents had them. These results are consistent with those of earlier LTF studies (Bradley, 1999). The omission of sampling sites other than the nostrils would have missed 12% (24 MSSA and one MRSA case) of the *S. aureus*-positive residents. Perirectal- perianal cultures have been shown to detect MRSA with high sensitivity in certain patient populations (Muto et al., 2003), although the cost effectiveness has shown little support for perianal screening in some cases (Papia et al., 1999). On the other hand, Coello and colleagues reported that in non-infected asymptomatic carriers, the perineum swab alone or in combination with a nose swab was more sensitive than the throat swab alone or in combination with a nose swab (Coello et al., 1994). Two recent studies, however,

concluded that samples taken from the throat should be included in screening patients for MRSA (Meurman et al., 2005; Ringberg et al., 2006). Nevertheless, collecting throat swabs from elderly persons suffering from dementia can be troublesome, and in the present study, the refusal rate with throat swabbing was the highest. Thus, swabbing the perineum while being washed could be a more comfortable way of sampling. The limitation in both of these studies was that only one screening round was carried out. The duration of MRSA carriage has been reported to last three to four months in LTFs (Bradley et al., 1991; Muder et al., 1991), but the status may have changed during the study, thus suggesting intermittent carriage (O'Sullivan and Keane, 2000b). Adequate differentiation between persistent and intermittent carriers would be relevant for epidemiological studies because of the increased risk for *S. aureus* infections with persistent carriers (Bruun, 1970). In addition, studies have shown bacterial genotypic variability to be lower for persistent carriers than for intermittent carriers, thus indicating that the underlying mechanisms that determine persistent and intermittent carriage differ (van Belkum et al., 1997).

6.2.2 The molecular epidemiology of MRSA and MSSA strains

Study III described the original MRSA outbreak consisting of isolates possessing a nationally new PFGE profile and internationally rare MLST type. In addition, three MRSA strains related to the FIN-7 profile were also detected. The outbreak of FIN-22 PFGE and ST-27 MLST types has been identified from only one Finnish patient outside this remote Finnish municipality, and the international MLST website showed that the MLST profile of that outbreak strain matched only three MSSA strains isolated from healthy carriers in England. However, no connections are known to have existed between these single individuals and those 13 residents in the LTF in Northern Finland. Among the 25 MSSA and 5 MRSA strains, 11 different PFGE patterns obtained from 24 different persons indicate the wide dissemination of different *S. aureus* genotypes in the small LTF population studied. In addition, two of the patients were colonized with *S. aureus* strains possessing two different MSSA and MRSA genotypes, and one of the patients was colonized with two different MSSA strains. According to Cespedes and colleagues, approximately 7% of *S. aureus*-colonized persons could be expected to carry more than one strain in their nostrils (Cespedes et al., 2005). A limitation of the present study was that only one MSSA strain per sample was studied, and therefore genotypically different MSSA strains from the same patient may have been missed. In addition, the nursing staff was not screened, so it cannot be known whether they carried the same genotype of *S. aureus*. 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, and especially to the FIN-10 and FIN-14 strains. The present data from a Finnish study revealed that despite the rather wide genomic diversity, most clinical MSSA isolates share identical genotypes with the non-multiresistant Finnish epidemic MRSA strains, including the FIN-7 strain (Ibrahem et al., 2005).

All the MRSA strains found in this survey shared identical SCC*mec* V, indicating that the same mobile genetic element has been integrated into the *S. aureus* chromosome of the strains in the patients. Among the epidemic strains in Finland, two other strain types, FIN-10d and FIN-17, also share SCC*mec* type V. FIN-10d has spread in Northern Finland and is resistant mainly to beta-lactams. Recently, at least one more strain type possessing SCC*mec* V has been identified in Finland (unpublished data). SCC*mec* V is reportedly to associated with community acquisition and distributed among coagulase-negative staphylococcal species, especially *S. haemolyticus* (Ito et al., 2004). Some have speculated that the *mec* gene complex and *ccr* gene complex of SCC*mec* go over complex rearrangement and recombination processes in coagulase-negative staphylococci and only some of them transfer to *S. aureus* (Grundmann et al., 2006; Ito et al., 2004). It would be interesting

to know whether the coagulase-negative staphylococci of the patients possessed the same SCC*mec* type V.

6.3 Laboratory diagnostics of MRSA

6.3.1 Culturing of *S. aureus* and MRSA

In Finland, ORSAB medium was used in more than half of laboratories in screening for MRSA directly from the routine clinical samples in 2001. ORSAB was used as a selective medium in both of the LTF studies carried out, and the results suggest that the medium was insensitive to the isolates expressing low-level oxacillin resistance, and was highly non-specific. ORSAB with 6 mg/l oxacillin, as CLSI recommends for screening, proved to be a valuable method for MDR, but may miss part of NMDR MRSA (Merlino et al., 2002b). According to the same study, if the oxacillin concentration is lowered to 2 mg/l, the ORSAB medium will also give a good correlation with NMDR (Merlino et al., 2002b). However, the ORSAB used in both of the present studies contained only 2 mg/l of oxacillin. Poor growth may be due to a very low amount of MRSA in specimens, or to inhibition by the high salt concentration (Van Enk and Thompson, 1992). In addition, almost one third of screening specimens obtained from NH in the Helsinki metropolitan area grew in ORSAB, which developed blue colonies, but only 1% of those were MRSA. Half of those blue-colony-growing bacteria were isolated from perineal swabs, and nearly all comprised coagulase-negative staphylococci (data not shown). The specificity of ORSAB decreased after 24 h of incubation, but to detect as well the MRSA strains that express very low-level oxacillin resistance, it is advisable to incubate the plate for as long as 96 h. Others have also reported problems with the specificity of ORSAB (Becker et al., 2002; Blanc et al., 2003). The percentages of sensitivity (60-67%) and specificity (study IV: 93% after 24 h, and 72% after 96h, respectively) obtained in these studies are low, but the results are likely to be a bit misleading than those of other works, because only three MRSA were found in study IV, and five MRSA in study III. Other commercial MRSA-selective media have been introduced recently, and have proven to perform with relatively good sensitivity and specificity (Compernolle et al., 2007; Flayhart et al., 2005; Louie et al., 2006; Loulergue et al., 2006; Pape et al., 2006; Perry et al., 2004; Stoakes et al., 2006), even with perineal swabs (Perry et al., 2004).

An additional 5% of *S. aureus* isolates were detected with enrichment broth in the study IV. This is less than that reported in other studies (Blanc et al., 2003; Safdar et al., 2003; van Ogtrop, 1995). However, one of the three MRSA isolates would have been missed without enrichment broth. Since only 0.6% of *S. aureus* isolates were found in a primary culture, it may be suggested that a primary culture is not necessary if a proper enrichment culture will be done. In this study, using enrichment broth was most effective in finding skin lesion isolates, followed by perineum isolates. Some of the screening sites were highly colonized with gram-negative rods, especially the perineal swabs with the swarming *Proteus* species (data not shown). Being aware of this problem, CLED agar was used in primary culturing or subculturing. The addition of antimicrobials, such as azreonam or nalidixic acid, to the broth would also have prevented this contaminating growth. A limitation of the study III was that *S. aureus* was screened without enrichment broth. Several studies have reported that one cannot avoid the use of an enrichment broth without a loss of sensitivity in the MRSA screening (Blanc et al., 2003; Safdar et al., 2003; van Ogtrop, 1995), although contradictory results do exist (VandenBergh et al., 1999).

6.3.2 Phenotypic detection of methicillin resistance in *S. aureus*

There was a wide variation in the MRSA screening practises used in Finnish microbiology laboratories, and especially the incubation times and incubation temperatures, vary widly and were not uniform. This may cause problems, especially in the detection of oxacillin-resistant-only isolates, heterogeneously resistant isolates, and isolates expressing low-level oxacillin MICs. The oxacillin disk diffusion test may miss part of the MRSA strains if the incubation time is not 24 h, as guided by CLSI recommends. The recommended incubation temperature for the oxacillin test is 35°C, but some of the heterogeneously resistant isolates are thermosensitive, and changing the temperature to 30°C may sometimes result in a large proportion of cells appearing resistant (Brown and Kothari, 1974; Merlino et al., 2002b). In the study V for evaluating phenotypic methods, decreasing the temperature failed to increase the sensitivity. On the contrary, sensitivity decreased, especially with cefoxitin. The cefoxitin disk would have missed a total of eight isolates out of 150, all of which were incubated at +30°C, and four of those with the salt addition. The performance of cefoxitin was, however, better than both oxacillin disk diffusion, and the oxacillin E-test, which proved to be the most non-specific. Other studies have evaluated the cefoxitin (Cauwelier et al., 2004; Felten et al., 2002; Skov et al., 2003; Skov et al., 2006; Swenson and Tenover, 2005) and moxalactam (Felten et al., 2002) disk diffusion tests in both MH and Iso-Sensitest agar, and reported better performance than that of the oxacillin disk. Two studies (Cauwelier et al., 2004; Felten et al., 2002) demonstrated the advantage of using an incubation temperature of 30°C with cefoxitin disk diffusion test, whereas a recently published study (Skov et al., 2006) showed no advantage to changing the temperature. The amount of salt added to the agar base varied from 0% to 5% in Finnish microbiology laboratories. Studies have reported that the addition of up to 5% NaCl to the test media improves the detection of resistance (Brown and Yates, 1986; Milne et al., 1993; Milne et al., 1987), but some strains are adversely affected by NaCl (Brown and Yates, 1986; Bruins et al., 2007). As recommended by the CLSI, 2% NaCl supplement in MH agar was used which, in the present material, seemed to improve the detection of *mec*A resistance only with the sensitivity of oxacillin E-test. All of the missed MRSA strains belonged to internationally known MRSA clones that are known to express oxacillin resistance very heterogeneously. In addition, half of the Finnish clinical microbiology laboratories used the latex agglutination test to detect the PBP2a protein. In general, this test is very sensitive and specific to *S. aureus* (Brown and Walpole, 2001; Cavassini et al., 1999; Sakoulas et al., 2001; Swenson et al., 2001; van Griethuysen et al., 1999), although it may prove unreliable for colonies grown on a medium containing NaCl (Brown and Walpole, 2001) or may require a longer agglutination period than what the manufacturer recommends (Swenson et al., 2001).

6.3.3 Genotypic verification of MRSA

Only one clinical laboratory in Finland used a PCR test to verify oxacillin-resistance in 2001. The sensitivity and specificity of phenotypic methods do not compare with genotypic methods, and thus the genotypic methods remain the gold standard for confirming MRSA. Since the KTL concluded in 2005 the verification of MRSA as a routine method and focused on molecular typing, the number of laboratories using molecular techniques has risen. In addition, such commercial diagnostic tests have increasingly come to the market, thus making verification easier. Due to this, a total of 12 clinical microbiology laboratories were using some sort of molecular application for verification of MRSA in 2006 (Anni Virolainen-Julkunen, personal communication). Two commercial molecular-based methods were tested for verifying MRSA and they were compared to two in-house PCR methods.

GenoType® MRSA and EVIGENE™ MRSA Detection tests were found over twice as fast and simpler than in-house PCR systems; thus these methods are suitable for confirming MRSA routinely in clinical microbiology laboratories. Cuny and colleagues have reported 100% sensitive and specific results for detecting MRSA in comparison to in-house *mecA-nuc* PCR with the former version of the GenoType® MRSA test (Cuny et al., 2001). However, many coagulase negative staphylococcal species, especially *Staphylococcus epidermidis*, possess the *mecA* gene (Petinaki et al., 2001), which may confuse the detection of MRSA. The improvement on the present version of GenoType® MRSA is the addition of a gene probe specific to *Staphylococcus epidermidis*. The GenoType® MRSA test correctly identified all the examined strains of *Staphylococcus epidermidis* (and *mecA* gene, if present), and no cross-reactions with other staphylococcal species were detected. Skov and colleagues have also reported that their results with the EVIGENE™ MRSA Detection test were in total agreement with the results obtained by both Southern blot and PCR methods (Skov et al., 1999).

Microbiological testing is still based largely on culturing, but rapid commercial tests directly from specimens have become available. These tests are promising (de San et al., 2007; Desjardins et al., 2006; Francois et al., 2007; Holfelder et al., 2006; Huletsky et al., 2005; van Hal et al., 2007; Warren et al., 2004), although, as presented recently, their clinical quality performance is yet far from optimal (van Belkum et al., 2007). In addition, some of them have been reported to miss MRSA strains possessing new SCC*mec* types or non-typeable strains (Francois et al., 2007), and therefore should be developed to identify the new SCC*mec* types as well.

7. CONCLUSIONS

The key findings in this present study are:

I. The rates of MRSA and screening practices varied widely across geographic regions. NMDR MRSA isolates, especially those resistant only to oxacillin/methicillin, showed an emerging trend. Part of these MRSA strains could remain undetected in some laboratories because of current diagnostic techniques used. The increasing proportion of elderly population carrying MRSA suggests that MRSA is an emerging problem in Finnish long-term facilities.

II. The trend in MRSA incidence was rising and the predominant MRSA strains changed over time and place, but two internationally spread epidemic strains of MRSA, FIN-16 and FIN-21, were related to the rise detected most recently. Those strains were also one cause of the striking increase in the number of invasive MRSA findings, thus showing the real worsening situation in Finland. The most common strains found from clinical specimens of the patients were the FIN-16 and FIN-4 strains, and from the screening specimens, FIN-7. The rise of MRSA strains with SCC*mec* types IV or V, possible CA-MRSA, was also detected.

III. The original MRSA outbreak in LTF, which consisted of isolates possessing a nationally new PFGE profile (FIN-22) and internationally rare MLST type (ST-27), was confined. Another previously unrecognized MRSA strain was found with additional screening, possibly indicating that current routine MRSA screening methods may be insufficiently sensitive for strains possessing low-level oxacillin resistance. Most of the MSSA strains found were genotypically related to the epidemic MRSA strains, but only a few of them had received the SCC*mec* element, and all those strains possessed the new SCC*mec* type V.

IV. Combining the use of enrichment broth and perineal swabbing, in addition to swabbing nostrils and skin lesions, could be an alternative to throat swabs in nursing home settings, especially when residents are uncooperative.

V. The cefoxitin disk diffusion method is superior to the oxacillin disk diffusion and MIC tests in predicting *mecA*-mediated resistance in *S. aureus* when incubating at +35°C. Both the Geno Type® MRSA and EVIGENE™ MRSA tests are usable, accurate, cost-effective, and sufficiently fast methods for rapid MRSA confirmation from a pure culture.

8. FUTURE CONSIDERATIONS

The future probably holds the never-ending battle with smirking *S. aureus*. In recent years, MRSA clones have emerged that are particularly fit for survival and spread beyond health care settings. In contrast to HA-MRSA clones, these CA-MRSA clones are often resistant only to beta-lactam antibiotics and may express resistance heterogeneously. Consequently, clinical microbiology laboratories may find it very challenging to detect these strains. According to our study, the proposed prevalence of CA-MRSA increased in Finland, although two internationally recognized hospital-acquired MRSA strain types were mainly responsible for the recent but geographically-restricted rise in MRSA rates. This new epidemiological situation with a community-associated MRSA strain is occurring worldwide, and should be studied more extensively in Finland. In addition, studying the virulence genes of strains that have caused severe infections would provide more information on the pathogenesis of MRSA strains circulating in Finland. Furthermore, sporadic strains have been reported to associate more often with community acquisition than with hospital acquisition, and it certainly may prove interesting to characterize these strains with greater specificity than that provided by mere typing with PFGE. Further studies are also needed to discover why some MSSA strains integrate *mecA* while other strains do not, and to investigate the SCC*mec* region of CoNS to get more information about the interspecies transfer of genetic information.

Since the elderly population in Finland is increasing, thus placing a greater burden on the healthcare system, the number of patients presumably bringing MRSA into hospitals with them will also increase. Controlling MRSA in long-term facilities is therefore important, but it faces with special challenges because it deals with a setting considered the resident's own home. It is necessary to make major surveillance efforts to detect the extent of the problem, and to introduce measures to prevent and control outbreaks in long-term facilities as much as in hospital settings. Politicians and healthcare administrators play a key role in providing the necessary resources for the problem. The Ministry of Social Affairs and Health in Finland allocated additional funds for MRSA prevention for the years 2005-2006; thus far, this special investment may have paid off because the rising incidence of new MRSA cases seems to be slowing. The most permanent trends in the near future, however, remain to be seen.

The importance of commercial methods in the identification of MRSA isolates will probably increase in the future, and especially that of screening assays to detect MRSA directly from the clinical specimens. In addition to two PCR-based methods, and a system that employs bioluminescence to detect the adenylate kinase of viable MRSA cells recently described, an important area for the application of chip arrays will emerge. Chip arrays, which have been used mainly in research setting (Koessler et al., 2006), would likely be developed for the rapid screening of bacterial isolates for both identification and antimicrobial resistance gene detection in clinical microbiology laboratories. At present, however, using a small chip for only one screening swab to detect MRSA may not be cost-effective.

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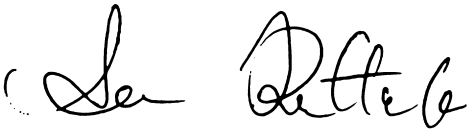

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