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**IN THE BEGINNING OF AN EPIDEMIC: EPIDEMIOLOGY OF METHICILLIN  
RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE SOUTH PART OF  
STOCKHOLM 2000 – 2003**

I BÖRJAN AV EN EPIDEMI: EPIDEMIOLOGI AV METICILLIN-RESISTENTA  
*STAPHYLOCOCCUS AUREUS* I SÖDRA DELEN AV STOCKHOLM 2000 – 2003

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## SAMMANFATTNING

Antalet patienter infekterade/koloniserade med meticillinresistent *Staphylococcus aureus* började stiga i den södra delen av Stockholmsområdet år 2000. Den här studien beskriver epidemin under åren 2000 – 2003. Analysen är baserad på information om de 181 nyupptäckta patienterna med MRSA, resultat av resistensbestämning mot antibiotika, molekylär epidemiologisk typning med pulsfälts gel elektrofores (PFGE) och multilocus sequence typing (MLST), och påvisande av Panton-Valentine leukocidin (PVL) gener. Ingen enskild MRSA klon orsakade epidemin. I stället ägde ett antal relativt små och begränsade utbrott rum, orsakade av olika MRSA kloner, och ofta utgående från en patient som hade förvärvat MRSA utomlands. Utbrott på sjukhus och i samhället orsakades av skilda kloner och pågick oberoende av varandra. De största utbrotten som drabbade sjukhuspatienter orsakades av sekvenstyperna (ST) 22, 239, 247, 8 och 45, och många av dessa isolat var multiresistenta mot antibiotika. Misstänkt heteroresistens mot glykopeptider identifierades hos isolat tillhörande ST 247, 239 och 592. I samhället var ST 80 den mest spridda, men även isolat tillhörande ST 8, 30, 59 och 150 var samhällsförvärvade. Dessa isolat var inte multiresistenta. De samhällsförvärvade klonerna var till skillnad mot de sjukhusförvärvade ofta positiva för PVL gener. Ett undantag var isolat tillhörande ST 150 som spreds i samhället bland hemlösa personer med sår på fötterna och traumatiska sår.

## ABSTRACT

The number of patients infected/colonised by methicillin-resistant *Staphylococcus aureus* (MRSA) began to rise in the southern part of the Stockholm area in 2000. The present study describes the epidemic during 2000 – 2003. The analysis is based on information concerning the 181 newly detected patients with MRSA, results of antibiotic susceptibility tests, and molecular epidemiologic typing with pulsed-field gel electrophoresis and multilocus sequence typing, and detection of Panton-Valentine leukocidin genes. No single MRSA clone was causing the epidemic. Instead a number of rather small, limited outbreaks took place, caused by different MRSA clones, and often starting from a patient who had acquired MRSA abroad. Outbreaks in the hospitals and in the community were caused by different clones and took place independent of each other. The largest outbreaks affecting hospital patients were caused by sequence types (STs) 22, 239, 247, 8 and 45, and many of these isolates were multiresistant to antibiotics. Suspected glycopeptide heteroresistance was found in isolates belonging to STs 247, 239 and 592. In the community the most widely spread MRSA was ST 80, but also isolates belonging to STs 8, 30, 59 and 150 were community acquired. These isolates were not multiresistant. In contrast to the clones transmitted in hospitals, the community acquired MRSA clones were often harbouring the PVL genes. One exception was isolates belonging to ST 150 which were transmitted in the community among homeless people with foot ulcers and wounds.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has during the past three decades evolved as a major cause of hospital-acquired infections in most countries around the world (44). This is mainly due to the clonal dissemination of a limited number of MRSA clones between cities, countries and continents (13, 28). In Sweden the prevalence of MRSA among invasive isolates of *S. aureus* has for a long time remained relatively low compared with other European countries (37). However, a large outbreak involving 147 patients, caused by a single epidemic MRSA strain, United Kingdom E16 (= EMRSA-16), occurred in Gothenburg 1997–2001 (3). In Stockholm the prevalence of patients colonised or infected with MRSA began to rise in 2000. From January 1, 2000, MRSA carriage and disease was made notifiable according to the Swedish Communicable Disease Act. The epidemiology of MRSA in the whole of Sweden during the years 2000–2003 has recently been described (35).

At the Karolinska University Laboratory, Huddinge, clinical samples as well as screening samples for MRSA from the south part of Stockholm city and county are received and epidemiologic typing of isolated MRSA is being performed. The number of diagnosed MRSA-positive patients at this laboratory 1996–2005 is shown in Figure 1.

The present study comprise a detailed analysis of the epidemiology of MRSA in the south part of the Stockholm city and county 2000–2003, a period which constitutes a transition from a situation with few MRSA-cases/year into the beginning of an epidemic situation. The analysis is based on information concerning the patients, the results of antibiotic susceptibility tests, molecular epidemiologic typing and detection of Panton-Valentine leukocidin (PVL) genes.

## MATERIALS AND METHODS

Hospitals, primary health care and population. Karolinska University Laboratory, Huddinge, is the laboratory at Karolinska University Hospital, Huddinge, a teaching hospital with 1076 beds. The laboratory is serving not only this hospital but also two other acute care hospitals, Stockholm Söder hospital with 545 beds and Södertälje hospital with 251 beds, and in addition St Erik's Eye Hospital, with 30 beds, Ersta Hospital, with 400 beds including a large clinic for medical and surgical gastroenterology, and two hospitals for the elderly, Nacka and Dalen. Furthermore, all health-care centers located in the south part of Stockholm city and county are served by the laboratory. The total population in the area was 900057 on December 31, 2003.

Screening policy. At the beginning of the study period MRSA screening was performed as recommended by the Swedish Institute for Infectious Disease Control (6). Patients that had been admitted to a hospital or treated at any health care facility outside of the Nordic countries during the past six months were isolated in a single room upon admission, until screening cultures for MRSA were found to be negative. Samples were taken from the nose, perineum, and if applicable from the following sites which, if present, were considered as risk factors for MRSA colonisation: damaged skin (wounds, eczemas etc), tracheostoma, injection site for a needle or catheter, and urine from any indwelling catheter. In addition, staff who had been working in health care outside of the Nordic countries were cultured from the nose and from damaged skin. When cultivation disclosed MRSA an investigation was always initiated including MRSA culture of the other patients at the same department. In June 14, 2001, the first MRSA control program written specially for Stockholm was taken into practice (18). From now on MRSA screening was additionally performed on all patients who were

considered at risk for MRSA colonisation (i.e. those with risk factors, see above), irrespective if they had been abroad or not, each time they were admitted to a hospital. The number of MRSA screening cultures at our laboratory rose from less than 1000 cultures/year in 2000 to 13411 cultures/year in 2003.

#### Collection of patient data

Information concerning the patients including age and sex, site of infection, recent travel abroad, hospital care in Sweden, contact with any person known to be colonised with MRSA, place of work etc. were collected from the clinicians at the same time as advice was given concerning infection control measures. Data on previous hospitalization was also collected from a database. All hospital admissions in Stockholm are registered in a central database, and by accessing this database the number of admissions and the number of days as an in-patient at any hospital in Stockholm were retrieved for all newly diagnosed MRSA-positive patients, from exactly one year before the diagnosis of MRSA until the day of diagnosis. Information from this database was also used for analysis of where MRSA transmissions probably had occurred and for contact-tracing.

#### Identification of MRSA

New MRSA-cases were found through cultures taken on clinical indication as well as through screening and contact tracing. The isolates were collected from a variety of body sites, in most cases from wounds, ulcers, abscesses, anterior nares and urine, but also from miscellaneous sites such as perineum, eczemas, blood, ascites, sputum, tracheal aspirates, auditory meatus, and injection sites for a needle or catheter. In the beginning of the study period MRSA screening was performed by direct culture of swabs on blood agar and mannitol salt agar with 1 µg of oxacillin/mL (34). A selective broth and real time PCR assay for rapid screening and

identification of MRSA was developed at our laboratory (14), and this method was used in the routine laboratory from September 16, 2002. All isolates of MRSA were finally confirmed with PCR for the *mecA* and *nuc* genes (5). The first isolate from each newly detected subject with MRSA was tested for antimicrobial susceptibility and then stored at  $-70^{\circ}\text{C}$ .

Epidemiologic typing was performed at a later stage but as soon as possible.

Antimicrobial susceptibility testing. All isolates were tested by disk diffusion method according to the Swedish Reference group for Antibiotics (29). Selected isolates were screened for glycopeptide resistance by three methods, the Etest macromethod (38), the Brain heart infusion 6  $\mu\text{g}/\text{mL}$  vancomycin agar screen (9), and Mueller-Hinton 5  $\mu\text{g}/\text{mL}$  teicoplanin agar screen (10). Isolates that were positive in any of the screening tests were subject to MIC determination against vancomycin and teicoplanin by using the Etest method as recommended by the manufacturer (AB Biodisk, Solna, Sweden).

Pulsed-field gel electrophoresis. The first isolate of MRSA from each patient was investigated. PFGE of *Sma*I digests of chromosomal DNA was performed according to the HARMONY protocol (26). Lambda ladder PFG marker (New England Biolabs) and *Sma*I-digested *S. aureus* CCUG 41582 (=NTCC 8325) was included on each gel. The banding patterns were analysed visually and with the GelCompar II software (Applied-maths, Kortrijk, Belgium). The Dice similarity coefficient was used, and the patterns were clustered by the unweighted pair group method using arithmetic averages (UPGMA). Each unique banding pattern was given a designation.

Multilocus sequence typing. MLST was performed as previously described by Enright, M.C. et al (12). Sequences of the seven housekeeping genes were compared to known alleles from

the MLST database ([www.mlst.net](http://www.mlst.net)) and the allelic profiles (= sequence types, STs) were determined based on this database. MLST was performed on selected isolates of MRSA, with guidance from the results of PFGE. MLST was performed if a unique PFGE pattern was observed in isolates from at least two patients, indicating a possible transmission. In such cases one of the isolates with an identical PFGE banding pattern was chosen for MLST.

PVL detection. Panton-Valentine leukocidin (PVL) genes were detected by PCR as previously described (23). The first isolate of MRSA from each patient was investigated.

## RESULTS

The number of new MRSA-cases/year began to rise in the south part of Stockholm in 2000, and has continued to rise each year since then. The number of new cases/year 2000 – 2003 were 30, 41, 52 and 58, in total 181 new cases during the period, of which 102 were males and 79 females (Table 1). Based on data such as recent travel abroad, hospital care abroad, or onset of an infection when outside of Sweden, new MRSA-cases were classified as either acquired abroad or in Sweden. Thirty-two males and 17 females acquired MRSA abroad, and the mean ages of males and females, respectively, were 51 (range 3–92) and 50 years (range 1–82). Seventy males and 62 females acquired MRSA in Sweden, and the mean ages, respectively, were 58 (range 2–91) and 62 years (range 1–94). For the patients who acquired MRSA abroad the geographical region where MRSA probably was acquired is shown in Table 2. Among those who acquired MRSA in Sweden, four were members of hospital staff, 86 had stayed as patients in hospitals in the Stockholm county for at least one day (often longer) during the year preceding the detection of MRSA, whereas 42 had not stayed as

patients at any hospital in the Stockholm county during the year preceding the detection of MRSA.

PVL genes were detected in MRSA from 30 patients (17%), 15 males and 15 females, and 23 of those had acquired MRSA in Sweden. The mean ages of the males and the females with PVL positive MRSA were 28 and 33 years respectively. Twenty-two of the 23 patients with PVL positive MRSA had not stayed as in-patients at any hospital in the Stockholm county during the year preceding the first positive MRSA culture. In contrast, 85 of 105 (81%) of the patients with PVL negative MRSA acquired in Sweden had been hospitalized in the Stockholm county for at least one day during the preceding year (Figure 2). This difference in patient history was highly significant ( $p < 0.0001$ , two tailed Fishers exact test). The distribution of PVL positive and negative MRSA among patients with different length of hospital stay during the year preceding detection of MRSA is shown in Figure 3.

The number of isolates resistant to antibiotics among all 181 isolates were as follows: Ciprofloxacin 100 (55%), clindamycin 85 (47%), tetracycline 71 (39%), gentamicin 60 (33%), fusidic acid 37 (20%), rifampicin 36 (20%), and trimethoprim/sulfamethoxazole 24 (13%). The resistance rates were different among PVL positive and negative MRSA as shown in Figure 4. The PVL positive strains were often resistant to tetracycline (72%) and fusidic acid (52%), but susceptible to most other antibiotics. Among PVL negative isolates multiresistance was common. The antibiotic resistance among MRSA isolated from patients who had acquired MRSA in Sweden and who had been hospitalized in the Stockholm county for at least one day during the year preceding detection of MRSA is shown in Figure 5. Almost all, 85 of 86, of these isolates were PVL negative, and the resistance rates to many antibiotics were high.

Three screening methods were used for detection of glycopeptide resistance. Using the Etest macromethod 23 isolates (13%) were positive, and 20 of these, but no others (11%), were also positive using the teicoplanin agar screen. The vancomycin agar screen was negative for all isolates. The positive screening tests for GISA indicated that 23 isolates had decreased susceptibility and probably were heteroresistant to glycopeptides (hGISA). The MICs of vancomycin among the 23 isolates varied between 1 and 3 mg/L, i.e. none was resistant. The MICs of teicoplanin varied between 1 and 4 mg/L for 20 of the isolates, was 6 mg/L for two isolates and 12 mg/L for one isolate. The MIC breakpoint above which isolates of *S. aureus* are interpreted as resistant to teicoplanin differ between countries (39). In Sweden, according to SRGA (29), the MIC breakpoint for resistance is  $\geq 8$  mg/L, and accordingly one of the isolates was classified as resistant to teicoplanin.

Molecular typing using PFGE and MLST made it clear that no single clone of MRSA was causing the epidemic situation. Instead different clones of MRSA caused a number of small outbreaks. Most of the outbreaks were limited to involve less than 10 patients. In total 34 unique banding patterns were disclosed by PFGE. The patterns were sometimes closely related, with only 1 – 3 bands difference. Such closely related patterns represent the same clone, according to guidelines for interpretation of PFGE results (36). When representatives of each of the 34 PFGE patterns were subject to MLST a total of 15 ST were found. Cluster analysis of PFGE results with the GelCompar II software is shown in the dendrogram in Figure 6, as is also the ST corresponding to each PFGE pattern. Cluster analysis of PFGE results and MLST gave highly concordant result. MLST therefore can be used for grouping of PFGE patterns. The number of PFGE patterns with identical ST and the number of patients infected/colonised with each ST is shown in Table 3.

PVL genes were always confined to all isolates of a certain PFGE pattern, and PVL therefore seemed to be a stable genetic marker in this collection of MRSA. PFGE patterns positive for PVL genes were 01-02, 01-06, 01-08, 01-04, and 01-05. The corresponding STs are shown in Figure 6. Isolates with one of the four PFGE patterns of ST 8 were PVL positive, as were isolates with one of the two PFGE patterns of ST 30, and both PFGE patterns of ST 80.

A detailed description of the STs and PFGE patterns of greatest importance for the MRSA epidemiology in Stockholm during the study period is given below. In the Harmony project epidemic MRSA strains from different European countries were collected and PFGE was performed (26). The designation of PFGE patterns used in the Harmony project is given when applicable.

ST 22. The largest outbreak was caused by ST 22, including three very similar PFGE patterns, 97-01, 00-00 and 02-08, and involving a total of 25 patients (17 males and eight females) who acquired MRSA in Stockholm 2000 – 2003. Two patients acquired ST 22 abroad. PFGE pattern 97-01 is identical to United Kingdom E15 in the Harmony project, and first appeared in Stockholm in 1997. The outbreak took place mainly in the north part of Stockholm (not described in the present study), and the south part experienced just a minor part. All the isolates were negative for PVL genes. Resistance to antibiotics among the 25 isolates were as follows: Ciprofloxacin 24 (96%), clindamycin 17 (63%), tetracycline 0 (0%), gentamicin 1 (4%), fusidic acid 3 (12%), rifampicin 1 (4%), and trimethoprim/sulfamethoxazole 0 (0%). The transmissions occurred principally among hospitalized patients, in most cases among old patients with ulcers and other risk factors for MRSA colonisation. Acute care hospitals, rehabilitation clinics and long-term care facilities were affected, as were homes for elderly.

ST 247. All isolates with ST 247 had the same PFGE pattern, 99-01, which is identical to Spain E1 in the Harmony project. Two patients acquired ST 247 abroad. The outbreak in Stockholm started in December 1999 with one of these patients as the source, and involved in total 12 medical intensive care patients (seven males and five females) and one staff (male) at Stockholm Söder hospital. PVL genes were not detected. The hGISA screening test was positive for 12 of the 13 isolates (92%). Resistance to antibiotics were as follows: Ciprofloxacin 13 (100%), clindamycin 13 (100%), tetracycline 13 (100%), gentamicin 13 (100%), fusidic acid 3 (23%), rifampicin 13 (100%), and trimethoprim/sulfamethoxazole 0 (0%).

ST 8. Isolates with PFGE pattern 00-01 were acquired by two patients abroad, transmitted to six hospital patients in Stockholm (five males and one female), and later to the husband of one of the MRSA carriers who was infected at home. This PFGE pattern is identical to France A in the Harmony project. PVL genes were not detected. Resistance to antibiotics among the seven isolates transmitted in Stockholm were as follows: Ciprofloxacin 4 (57%), clindamycin 4 (57%), tetracycline 0 (0%), gentamicin 0 (0%), fusidic acid 1 (14%), rifampicin 2 (29%), and trimethoprim/sulfamethoxazole 0 (0%).

PFGE pattern 01-02 was acquired from one patient abroad and transmitted between seven patients in Stockholm (five males and two females) who had not been hospitalized during the year preceding detection of MRSA. The common factor was that those affected were either wrestlers or sexual partners to wrestlers. All isolates were positive for PVL genes. All isolates transmitted in Stockholm were resistant to tetracycline, but none of them were resistant to any other antibiotic tested except methicillin.

ST 239. Nine different PFGE patterns were represented among isolates with ST 239 (Figure 6), and altogether 22 patients (16 males and six females), seven (six males and one female) of whom acquired MRSA abroad. Epidemiological information revealed a number of small, limited outbreaks, each involving 1 – 3 hospitalized patients, at different hospitals. Four ST 239-isolates were positive in hGISA screening. The corresponding patients were two with PFGE pattern 01-10 who were infected abroad, and two with PFGE pattern 01-03 who were infected from the same source at a hospital in the north part of Stockholm. PFGE pattern 02-04, which was acquired by one patient abroad, is identical to United Kingdom E1 in the Harmony project. Resistance to antibiotics among the 15 ST 239-isolates transmitted in Sweden were as follows: Ciprofloxacin 15 (100%), clindamycin 13 (87%), tetracycline 13 (87%), gentamicin 15 (100%), fusidic acid 9 (60%), rifampicin 10 (67%), and trimethoprim/sulfamethoxazole 11 (73%).

ST 45. Six hospital patients and three hospital staff (two of which were a couple), altogether four males and five females, were infected with MRSA of PFGE pattern 01-01, which is very similar to Berlin IV in the Harmony project. One of the isolates was resistant to fusidic acid, but otherwise the isolates were not resistant to the antibiotics tested except methicillin. Two patients with PFGE pattern 03-01 and one patient with PFGE pattern 01-07 had not been hospitalized during the year preceding MRSA detection and probably acquired MRSA in Stockholm. The PFGE pattern 03-01 isolates were resistant only to tetracycline and methicillin and the PFGE pattern 01-07 isolate was resistant only to methicillin. PVL genes were not detected among ST 45 isolates.

ST 88. Four of seven patients (two males and five females) with PFGE pattern 03-03 were living in homes for the elderly, three of them in the same home. They were probably infected

in their homes. PVL genes were not detected and none of the isolates were resistant to any other antibiotic than methicillin.

ST 150. All seven patients infected (six males and one female) were homeless people, and many of them were using intravenous drugs. MRSA was isolated from foot ulcers or wounds. All the patients had been hospitalized for short periods during the year preceding MRSA detection, but they probably had had more contacts with each other when staying over night at special hostels, and in the streets, and most probably the transmissions occurred outside of hospitals. The isolates were negative for PVL genes. Resistance to antibiotics, except methicillin, was found only in one isolate which was resistant to tetracycline.

ST 80. Isolates with PFGE pattern 01-04 dominated, five patients (two males and three females) acquired them abroad and nine (five males and four females) in Stockholm. Transmissions probably occurred within families in two cases (two different families). This PFGE pattern is known in many other European countries (42) including Denmark (15). PFGE pattern 01-05, which was very similar to 01-04, was acquired in Stockholm by two members of the same family (one male and one female). All ST 80 isolates were positive for PVL genes. None of the patients who acquired ST 80 isolates in Sweden had been hospitalized during the year preceding detection of MRSA. All of the 11 ST 80-isolates transmitted in Stockholm were resistant to fusidic acid and six were resistant to tetracycline. None of the ST 80 isolates were resistant to ciprofloxacin, clindamycin, gentamicin, rifampicin or trimethoprim/sulfamethoxazole.

ST 36. One patient was infected abroad with an isolate with PFGE pattern 00-05, which is identical to United Kingdom E16 in the Harmony project. Another patient was infected with

PFGE pattern 00-03 abroad, and four patients (one male and three females) acquired isolates with this pattern in Stockholm. All were residents in homes for the elderly and most probably a chain of transmissions did occur. All ST 36 isolates were negative for PVL genes. The four isolates acquired in Stockholm were all resistant to ciprofloxacin and clindamycin.

ST 59. Two patients (one male and one female) were infected with PFGE pattern 01-08. They had not been hospitalized during the year preceding detection of MRSA. The isolates were positive for PVL genes, and resistant to clindamycin and tetracycline, but not to other antibiotics except methicillin.

ST 5, ST 30, ST 231. These STs were isolated from only few patients (Table 3).

Unique PFGE patterns. Thirty-four isolates each had a unique banding pattern different from all other isolates. MLST was not performed. Many of these isolates (n = 23) were acquired abroad. Two of the 34 isolates were positive for PVL genes. They were both acquired in Stockholm, one by a patient who had stayed in a hospital for a single day during the year preceding detection of MRSA, and the other by a patient who had not been hospitalized during the previous year.

## DISCUSSION

The epidemiology of MRSA in the south part of Stockholm 2000–2003 is described in the present report. The period is of interest because a transition took place from a stable situation where few (9–22) patients with MRSA infection/colonisation were detected each year into the first phase of an epidemic situation. The number of new cases/year 2000 – 2003 were 30, 41,

52 and 58, in total 181 new cases during the period, and the yearly numbers have continued to rise since then (Figure 1). Forty-nine of the patients included in the study acquired MRSA abroad and 132 acquired MRSA in Sweden. The majority of all patients, 103 of 181 (57%), were males, and of those who acquired MRSA abroad 32 of 49 (65%) were males. A preponderance for males over females to acquire antibiotic resistant bacteria and MRSA have been reported by others, but the causes of excess resistance in males are obscure (24, 33). When MRSA was acquired abroad and subsequently imported to Sweden it was in most cases from other countries in Europe, 21 of 49 cases (43%), and otherwise all other inhabited continents except Australia were represented. This distribution mirrors the pattern of travel to and from Sweden.

Two methods, PFGE and MLST, were used for epidemiologic typing of MRSA. PFGE is, because of its high discriminatory power and its suitable stability over time, considered as a method of choice for the investigation of local outbreaks (36). The stability of PFGE results has been studied by analysing isolates recovered over time from long-term carriers of MRSA (4). The majority of consecutive isolates from the same patient, 88%, showed indistinguishable DNA banding patterns, but variants differing by one to six fragments was also isolated. In guidelines for interpretation of PFGE results it is stated that two isolates may be closely related if there is a difference of up to three bands, and may possibly be related if there is a difference of up to six bands (36). During a large and prolonged MRSA outbreak many variants of PFGE band patterns may arise within the same MRSA clone, as described for example from Spain where as many as 21 PFGE subtypes of an epidemic clone was reported (27). MLST is a method suitable for phylogenetic studies and world-wide epidemiological studies of MRSA (32). The stability of MLST is even higher than that of PFGE, which results in a lower discriminatory power and a decreased resolution compared to

PFGE (17, 40). Because of the clonal nature of *S. aureus* dissemination (16) typing methods like PFGE and MLST, each with a high stability, gives highly concordant result concerning the genetic relationship among isolates of MRSA (2, 17, 25, 30, 40). As this concordance has been demonstrated in other studies, only a selection of isolates were subjected to MLST in the present study. MLST was performed from only one chosen isolate among isolates with the same PFGE pattern isolated from two or more patients, and the resulting ST was obtained for all isolates having the same PFGE pattern.

The results of PFGE and MLST typing made clear that the rising number of patients with MRSA detected in the south part of Stockholm during the study period was not the result of an outbreak caused by a single epidemic MRSA clone. Instead a number of rather small, limited outbreaks took place, caused by different MRSA clones, and often starting from a patient who had acquired MRSA abroad. Many of the epidemic MRSA clones reported from different parts of the world to the MLST database ([www.mlst.net](http://www.mlst.net)) were represented, including clones belonging to all the five major lineages, or clonal complexes of MRSA, described by Robinson and Enright (32). Although each of the outbreaks in the south part of Stockholm was successfully terminated or confined by infection control measurements, more and more MRSA clones were introduced into the area and therefore it was a constant rise in the number of newly detected cases. Most trouble in the south part of Stockholm was caused by MRSA clones belonging to clonal complex 8, including ST 8, 239, 247 and 250. These clones were spread principally in hospitals among patients with risk factors for MRSA colonisation. A total of 36 hospitalized patients were infected/colonised with MRSA clones belonging to clonal complex 8 in the south part of Stockholm during the study. The single most abundant clone, however, was ST 22 belonging to clonal complex 22. This clone caused a large epidemic in the hospitals in the north part of Stockholm and was also spread to the south part.

In total 22 hospitalized patients, most of them with risk factors for MRSA colonisation, were infected/colonised by ST 22 in south Stockholm. ST 22 (= United Kingdom E15) is one of the most widely spread MRSA clones in United Kingdom (22).

Most of the isolates belonging to clonal complexes 8 and 22 were multiresistant to antibiotics. Resistance to ciprofloxacin was 100% among isolates transmitted in Sweden belonging to ST 247 and 239 and 96% in ST 22. Resistance to vancomycin was not found, but one isolate with teicoplanin MIC 12 mg/L was classified as resistant to teicoplanin according to SRGA (29). Twenty-three isolates had suspected heteroresistance to glycopeptides (hGISA) (7). To confirm if these isolates really are hGISA, population analysis is necessary to do (43), and this has not yet been done. MLST showed that 17 of the suspected hGISA belonged to clonal complex 8: 12 belonged to ST 247, four to ST 239 and one to ST 592. According to reports from Spain and France reduced susceptibility to vancomycin and teicoplanin has been found in isolates of MRSA with ST 247 (1, 19). The suspected hGISA isolates belonging to ST 247 detected in Stockholm might belong to the same clone. Heterogeneous GISA have been reported also in other MRSA lineages (21). There is controversy regarding the clinical significance of hGISA, but treatment failures have been described when treating serious infections caused by hGISA MRSA with vancomycin (20).

Clones of *S. aureus* producing PVL toxin have been associated with an increased tendency to cause skin infections (23). In 2002 it was reported from France that a clone of MRSA positive for PVL toxin genes was spreading in the community (11). In 2003 world-wide emergence of community-acquired MRSA carrying PVL genes was reported (41). This prompted us to perform a PCR assay to detect if isolates in the present study were harbouring the PVL genes. Forty-two patients with MRSA had not stayed as patients at any hospital in the Stockholm

county during the year preceding MRSA detection, and MRSA isolates from these were often positive for the PVL genes, 22 of 42 (52%). In contrast only one of the 85 isolates from patients who had been hospitalized during the preceding year was positive for the PVL genes, and this patient was hospitalized for only a single day. This difference in patient history is highly significant ( $p < 0.0001$ , two tailed Fishers exact test). PFGE and MLST typing showed that patients who had not been hospitalized were infected by other clones of MRSA than the clones transmitted in the hospitals. Multiresistant MRSA were isolated almost only from patients who had been hospitalized. When combining all this information it seems likely that some MRSA clones were community acquired and that MRSA epidemics in the community and in the hospitals occurred simultaneously and independent of each other.

The most widespread MRSA clone among community-acquired MRSA in the south part of Stockholm had PFGE pattern 01-04, ST 80. This clone, which is positive for PVL genes, has also been reported as a community acquired MRSA in other European countries (15, 42). Another PVL positive community acquired MRSA clone, PFGE pattern 01-02, ST 8, was transmitted in Stockholm between wrestlers and their sexual partners. This clone was probably brought to Sweden from South America by a Swedish traveller. A few PVL positive community-acquired MRSA had other sequence types: ST 30 was isolated from two persons in the same family, and ST 59 was isolated from one person who acquired MRSA abroad and from one who acquired MRSA in Sweden. Isolates with ST 150 were probably transmitted in the community but this clone was negative for PVL genes. The patients affected were homeless people, all of them had wounds and ulcers and they were therefore at risk for *S. aureus* infections. Foot ulcers are very common among homeless people (31), and MRSA has been previously reported among the urban poor of San Fransisco (8).

Since the end of 2003, the number of newly detected patients with MRSA has continued to rise each year in the south part of Stockholm (Figure 1), but still, in 2006, the prevalence of MRSA is relatively low compared with other countries. By infection control efforts the transmission of MRSA in the hospitals have largely been stopped (so far), but in the community there is an on-going transmission of community-acquired MRSA. The epidemiology of community-acquired MRSA in the south part of Stockholm will be described in a separate paper.

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Table 1. Number of newly diagnosed patients with MRSA at Karolinska University Laboratory, Huddinge 2000 – 2003.

Year	Acquired abroad		Acquired in Sweden		Total
	Male	Female	Male	Female	
2000	9	3	11	7	30
2001	3	6	18	14	41
2002	11	4	21	16	52
2003	9	4	20	25	58
Total:	32	17	70	62	181

Table 2. Geographical regions where MRSA acquired abroad probably were acquired.

<u>No. of MRSA cases *</u>	<u>Geographical region</u>
21	Europe
12	Asia
6	Middle East and Turkey
4	North America
4	South and Central America and the Caribbean
2	Africa

\* detected at Karolinska University Laboratory, Huddinge 2000 – 2003.

Table 3. Sequence type (ST) of all newly detected MRSA at Karolinska University Laboratory 2000 – 2003. In addition the number of PFGE patterns with a common ST is shown as well as the number of patients affected by each ST, and the patients are further divided into those infected/colonized abroad and those infected/colonized in Sweden. The latter group is subdivided into those hospitalized or not hospitalized during the year preceding the first positive MRSA culture. The number of staff infected/colonized with MRSA is also shown.

ST	No. PFGE-types	Total no. patients	No. patients who acquired MRSA abroad	No. patients who acquired MRSA in Sweden, >1 day hospitalization	No. patients who acquired MRSA in Sweden, no hospitalization	No. staff who acquired MRSA
8	4	20	4	8	8	0
239	9	22	7	15	0	0
247	1	15	2	12	0	1
250	1	2	0	1	1	0
592	1	2	2	0	0	0
5	2	4	0	2	2	0
231	1	1	1	0	0	0
30	2	4	1	2	1	0
36	2	6	2	3	1	0
45	3	12	0	6	3	3
22	3	27	2	22	3	0
59	1	2	0	0	2	0
150	1	7	0	7	0	0
80	2	16	5	0	11	0
88	1	7	0	2	5	0
NT	34, each with a unique banding pattern	34	23	6	5	0

Figure 1. The number of newly diagnosed MRSA patients/year at Karolinska University Laboratory, Huddinge 1996 – 2005. Black bars indicate the period of the present study (2000 – 2003).

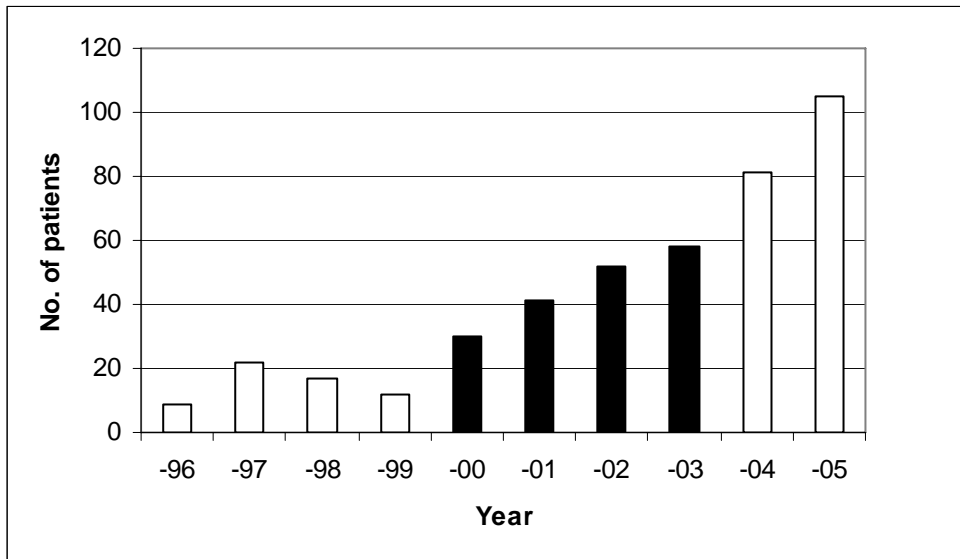


Figure 2. The association between previous hospitalization and infection/colonisation with MRSA harbouring Panton-Valentine leukocidin (PVL) genes.

		PVL		
		+	-	
Previous hospitalization	+	1	85	86
	-	22	20	42
		23	105	

Figure 3 The distribution of PVL positive and negative MRSA among patients who acquired MRSA in Sweden (n = 128) who had a history of different length of hospital stay during the year preceding first positive MRSA culture.

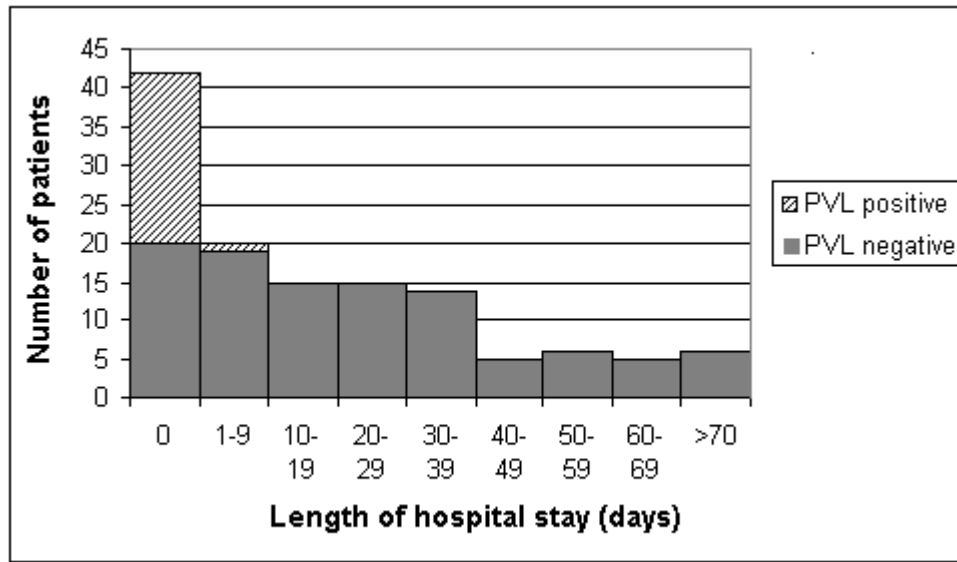


Figure 4. Antibiotic resistance rate among PVL positive (n = 30) and PVL negative MRSA (n= 151). TET = tetracycline, FUS = fusidic acid, CLI = clindamycin, CIP = ciprofloxacin, RIF = rifampicin, SXT = trimethoprim/sulfamethoxazole, hGISA = heteroresistant glycopeptide-intermediate *S. aureus*.

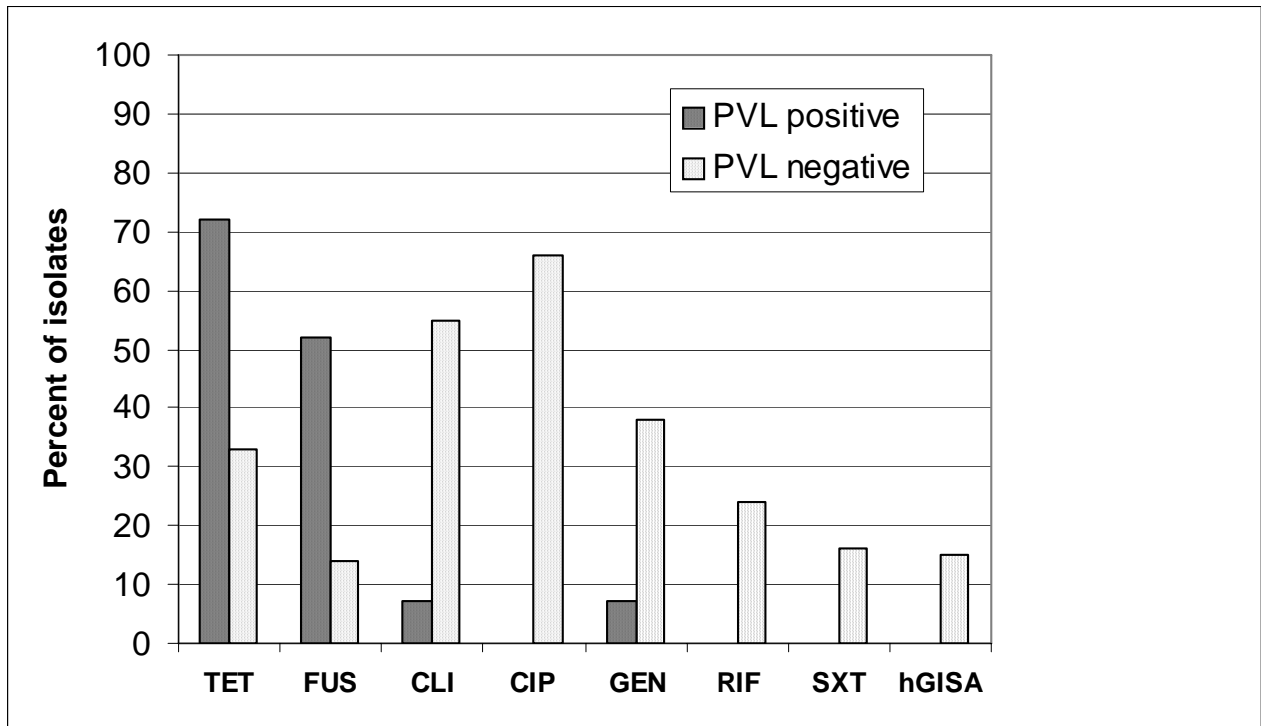


Figure 5. Antibiotic resistance rate among MRSA acquired in Stockholm by patients who had been staying at a hospital in the Stockholm county for at least one day (often longer) during the year preceding the first positive MRSA culture (n = 89). TET = tetracycline, FUS = fusidic acid, CLI = clindamycin, CIP = ciprofloxacin, RIF = rifampicin, SXT = trimethoprim/sulfamethoxazole, hGISA = heteroresistant glycopeptide-intermediate *S. aureus*.

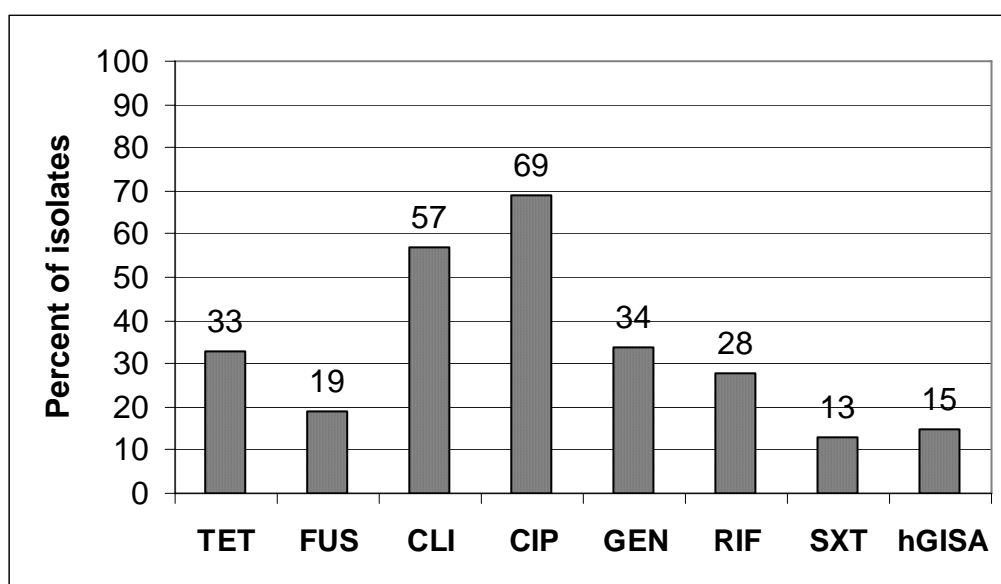


Figure 6. UPGMA dendrogram illustrating similarity (based on Dice coefficients) of macrorestriction profiles (*Sma*I PFGE) of 34 isolates of MRSA. Sequence type (ST) according to multilocus sequence typing is also shown.

