

Spread of a methicillin-resistant *Staphylococcus aureus* ST80 strain in the community of the northern Netherlands

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Abstract Infections caused by community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) are emerging as a major public health problem. In this study, we describe the distribution of 54 Panton-Valentine leucocidin (PVL)-carrying MRSA isolates in the northern Netherlands between 1998 and 2005, of which 43 (80%) consisted of the European PVL-positive strain multi locus sequence type 80 with staphylococcal cassette chromosome *mec* type IVc (ST80). Individual cases and small clusters of ST80 predominated in the community (74%), but ST80 was also found in nursing homes (16%) and hospitals (9%). Long-term carriage (months to years) and reinfection of patients with ST80 has probably led to the strain spreading in the community and subsequently to further migration to health care environments.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired infections and a serious public health concern [1]. In European hospitals,

high prevalences of MRSA (>40%) are reported in Greece, Ireland, UK, Italy and Malta, and low prevalences (<1%) in the Netherlands, Denmark, Sweden, Finland, Iceland and Estonia [2]. The low prevalence in Dutch hospitals can be explained by restrictive antibiotic use and our national “search-and-destroy” policy [3], which requires that patients who are repatriated from foreign countries, contacts of MRSA patients, citizens and patients from abroad are strictly isolated at hospital admission until screening cultures for MRSA prove negative. In case of MRSA carriage or infection, patients are kept in isolation and receive eradication therapy. The low prevalence of MRSA in hospital settings may be endangered by an increasing number of outbreaks of community-acquired MRSA (CA-MRSA) [4], resulting in migration of these strains to health care environments.

CA-MRSA infections mainly cause primary skin and soft tissue infections (SSTI) [5] or necrotising pneumonia in previously healthy patients. The majority of CA-MRSA strains harbour the Panton-Valentine leukocidin (PVL) genes, which encode for a bicomponent hymenotropic toxin responsible for pore formation in leucocytes, whereas most hospital MRSA strains do not. Other virulence factors associated with CA-MRSA are the superantigen staphylococcal enterotoxins (SE) B, C and H [1].

In the Netherlands, the prevalence of PVL-positive MRSA has increased over the last 5 years, and in 2004 the proportion of all MRSA isolates in the Netherlands that was PVL positive was 10% [6]. We surveyed all PVL-positive MRSA isolates collected in our laboratories over a period of 6 years and 8 months to describe the spread of the PVL-positive MRSA strain staphylococcal cassette chromosome *mec* type IVc (ST80) between 1998 and 2005, using molecular typing and retrospective review of available medical records.

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Materials and methods

We studied all PVL-positive MRSA isolates cultured between August 1998 and March 2005 in the northern Netherlands. The study area consisted of the Dutch provinces Groningen and Drenthe (5,639 km²; 1,058,407 inhabitants). All MRSA isolates were cultured at the Laboratory for Infectious Diseases in Groningen (37 isolates) and the laboratory of the Department of Medical Microbiology of the University Medical Centre, Groningen (17 isolates), which cover all general practitioners, nursing homes, outpatient clinics and hospitals of the region. Isolates were obtained from cultures of patients displaying typical staphylococcal disease syndromes (e.g. SSTI) or during routine MRSA screening as part of our national search-and-destroy policy (cultures of nose, throat and perineum). From each patient, only one PVL-positive MRSA isolate was included in the study. Clinical information regarding MRSA patients was obtained from their primary physicians by standardised questions.

Infections were classified as community acquired if isolates were obtained outside a hospital or nursing home setting or less than 48 h after hospital admission. In case of an MRSA infection involving personnel of a health care facility, acquisition was classified as community acquired when no previous contact with an MRSA-positive patient could be established. Foreign travel, hospitalisation or residence in a nursing home during the year before infection, outpatient visits and work in a care facility were considered risk factors for MRSA acquisition.

Patients positive for CA-MRSA underwent decolonisation therapy with mupirocin and Hibiscrub. Household contacts were screened for MRSA and received decolonisation therapy when they tested positive. After 3 weeks, the nose, throat and perineum were recultured for MRSA screening. Repetitive cultures were obtained according to the Working Group Infection Prevention (WIP) guidelines for MRSA [7]. Decolonisation therapy was continued if these cultures were MRSA positive and terminated if cultures were MRSA negative. In case of long-term carriage, patients were screened for MRSA with 3- to 6-week intervals and continued to receive decolonisation therapy until proven culture negative.

Colonies characteristic for *Staphylococcus aureus* were identified by DNase and tube coagulase tests. Susceptibility was tested by the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [8], against oxacillin (Oxoid B.V. Haarlem, the Netherlands), kanamycin, gentamicin, trimethoprim, trimethoprim-sulfamethoxazole, erythromycin, tetracycline, clindamycin, ciprofloxacin, rifampicin, vancomycin, mupirocin and fusidic acid (NeoSensitabs, Rosco, Taastrup, Denmark). Isolates were also screened for minimum inhibitory concentration (MIC)

of tetracycline, minocycline and fusidic acid by E-test and for inducible resistance to minocycline by tetracycline by double-disc diffusion [9]. Methicillin resistance was confirmed by the penicillin binding protein (PBP) 2' latex agglutination test (Denka Seiken, Tokyo, Japan). Additionally, the *mecA* gene was identified using the Genotype MRSA test (Hain Lifescience GmbH, Nehren, Germany).

DNA was extracted after prelysis with lysostaphin, as described previously [10]. Sequences specific for SEs A, B, C, D and E; exotoxin A (ETA); and toxic shock syndrome toxin 1 (TSST-1) were detected by polymerase chain reaction (PCR) using primers described previously [11]. Primer sequences used to amplify a 186 bp section of the SE-H gene were forward 5' GCAGTTGCAAACCTTACTCTCAAA 3', reverse: 5' CGAAAGCAGAAGATTACACGATA 3'. Primer sequences used to amplify a 530 bp section of the PVL gene *lukS-PV* were forward 5' ATGACTCAGTAAACGTTGTAGAT 3', reverse 5' TCTATCCATTTCACTTTGATAAGT 3'. Primer sequences used to amplify a 305 bp section of tetracycline resistance determinant tet K were forward 5' ATGTGCTATTCCTCCCTATTGA 3', reverse 5' TCGATAGGAACAGCAGTATATGGA 3'. Primers for amplification of a 385 bp section of the Nuc gene (specific for *S. aureus*) were added as a control in all PCR runs: forward 5' CGCTAC-TAGTTGCTTAGTGTT 3', reverse 5' CACGTCCAT ATT-TATCAGTTC 3'. All primers were developed using Primer3. PCR products were resolved by electrophoresis with 1% agarose gels, which were stained with ethidium bromide and analysed. SCC*mec* typing was initially performed by Ito on two isolates and resulted in SCC*mec* type IVc, after which all isolates were tested for SCC*mec* type IVc [12].

MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE) after *Sma*I macrorestriction, and PFGE patterns were interpreted according to criteria proposed by Tenover et al. [13]. PVL-positive MRSA isolates in the follow-up cultures were not subjected to PFGE analysis. These isolates were considered identical if the PBP2' latex agglutination test was positive and resistance patterns were similar. Furthermore, all MRSA isolates were sent to the National Institute of Public Health and Environment (RIVM; Bilthoven, the Netherlands)—which serves as the national reference centre for surveillance of MRSA in the Netherlands—for verification of possession of *mecA* and PVL genes and for additional typing.

Results and discussion

From August 1998 to March 2005, 54 PVL-positive MRSA isolates were cultured in our laboratories. A total of 22% of all MRSA isolates in the northern Netherlands were PVL

positive, being twice as high as the Dutch national average of 10% in the same time period [6]. Multilocus sequence typing (MLST) characterised ST80 as the predominant PVL-positive MRSA strain in the Netherlands, covering 20% of all PVL-positive MRSA isolates [6]. In the northern Netherlands, 43 of the 54 PVL-positive MRSA isolates (80%) were characterised as PFGE cluster 28 (RIVM), previously identified by MLST as the ST80 strain [6]. Data were collected using the same sample frame and comparable methodology.

The first appearance of the ST80 strain in the northern Netherlands was in 1998, and since 2002, the ST80 strain was identified repeatedly in the northern Netherlands. Individual infections and small clusters (involving two to five patients) of infection or colonisation with ST80 occurred 29 times, without apparent patient contacts between these individual cases or clusters. Patients of all ages harboured the ST80 strain (median age 48 years; range 0–91). Apart from the ST80 strain, seven different PVL-positive strains as determined by PFGE (not shown) were cultured from 11 patients. Identical strains in this “non-ST80” group were found only within families.

All ST80 isolates contained genes for *mecA*, *tetK*, PVL and SEH. SCC*mec* type IVc was confirmed in 42 of the 43 isolates (98%). All ST80 strains were susceptible to gentamicin, trimethoprim, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ciprofloxacin, rifampicin, vancomycin and mupirocin. Apart from resistance to beta-lactam antibiotics and kanamycin, all ST80 isolates showed resistance to fusidic acid (MIC range 8–16 µg/ml) and tetracycline (MIC range 16–24 µg/ml). Since all isolates were susceptible to minocycline (MIC range ≤0.125 µg/ml)

without induction of resistance by tetracycline, resistance to tetracycline was determined only by *tetK*, without interference of *tetM*. Acquisition of fusidic acid and tetracycline resistance by ST80 strains in the community may be due to the frequent subscription of fusidic acid or tetracycline by general practitioners to patients with SSTI [14]. In other European studies regarding the ST80 strain, tetracycline- and fusidic-acid-resistant and susceptible strains were described, as well as additional resistance to ciprofloxacin, erythromycin or chloramphenicol and differences in the presence of toxins [1]. Although the ST80 strain is considered to be a single European strain, mutations and different antibiotic regimens between countries may increasingly lead to the development of heterogeneous strains.

Table 1 shows the assumed location of acquisition of the PVL-positive MRSA isolates. The majority of ST80 isolates (32 of 43, 74%) was acquired in the community, of which 16 (37%) were isolated from patients without risk factors for MRSA acquisition. Nine ST80 isolates (21%) were recovered from community patients with risk factors for MRSA acquisition, consisting of hospitalisation or outpatient visits in the year before infection. However, as no MRSA infections were registered in the hospital wards or outpatient clinics during admittance or visits of any of these patients, acquisition of the ST80 strain may still have been truly community acquired. Nevertheless, prior health care exposure could have predisposed these patients to become colonised with a community-dwelling MRSA strain due to antibiotic use or chronic illness [15]. Seven ST80 isolates (16%) were derived from household contacts (predominantly colonisation), five originating from true

Table 1 Location of acquisition and initial source of isolation of all 54 Panton-Valentine leucocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* isolates [staphylococcal cassette chromosome *mec* type IVc (ST80) and non-ST80] cultured between 1998 and 2005 in the northern Netherlands

	Location of acquisition	Number (%)	Source of isolation		
			SSTI	Colonisation	Other
ST80 (n=43)	Community				
	no risk factors	16 (37)	13	1	2
	risk factors	9 (21)	6	2	1
	household	7 (16)	3	2	2
	Nursing home	7 (16)	6	1	–
	Hospital	4 (9)	–	3	1
Non-ST80 (n=11)	Community				
	no risk factors	2 (18)	2	–	–
	risk factors	4 (36)	2	1	1
	household	4 (36)	–	4	–
	Nursing home	1 (9)	1	–	–

SSTI skin and soft tissue infections.

CA-MRSA strains and two from family members of a nursing home nurse, all three of them suffering furunculosis by ST80. As only one nursing home patient in this small outbreak was infected by ST80, it remained unknown which individual was infected first and served as the index patient. Four ST80 isolates (9%) were acquired in a hospital. However, three of these hospital isolates could be directly linked to the transfer of a nursing home patient suffering a severe wound infection by ST80 to the hospital, colonising two patients and a hospital nurse. Although seven (16%) isolates were defined to be acquired in a nursing home, in total, 28% of all ST80 isolates had a link to a nursing home, making nursing homes a potential intermediate for transmission of MRSA from the community to the hospital. The majority (91%) of PVL-positive non-ST80 isolates was also community acquired (Table 1), although six strains (55%) involved adoption of children from China, where the incidence of MRSA is high [1].

Most ST80 isolates, 28 of 43 (65%), were initially isolated from various SSTI, predominantly wound infections, abscesses and furunculosis (Table 1). Nine patients (21%) displayed no symptoms but MRSA screening revealed colonisation with ST80 after contact with MRSA-positive patients, most often household members. ST80 was also obtained from cultures of several other infections, including pneumonia, otitis media, conjunctivitis (in two neonates) and sepsis, the latter resulting in the death of one patient within 24 h after hospitalisation. The 11 PVL-positive non-ST80 isolates originated equally from SSTI (five strains, 45%) or colonisation (45%), whereas one was isolated from a patient with pneumonia.

Despite eradication therapy with a combination of mupirocin, Hibiscrub and oral antibiotics (trimethoprim-sulfamethoxazole, macrolides or clindamycin) in case of invasive infection, a number of patients were found to be carrying MRSA ST80 on follow-up screening cultures. Control MRSA screening revealed that in 22 patients (51%), one eradication treatment was successful within a month. Several patients were shown to continue carrying the MRSA strain for 2–3 months (seven patients, 16%), 6 months (four patients, 9%) or for over a year (ten patients, 23%). In one family, ST80 carriage for more than 2 years was demonstrated by 3- to 6-monthly MRSA screening of a young infant and her mother, the latter suffering serious SSTI by ST80 2 years after initial colonisation. The ST80 carriage in this family led to ST80 colonisation of the second child at birth and SSTI by ST80 in another adult relative of this family, without existing household membership.

The high occurrence of ST80 in the northern Netherlands can probably be ascribed to local spread of the ST80 strain in the community, where most strains were acquired. Because there is no national policy for general practitioners

for handling a CA-MRSA infection, adequate MRSA screening of all contacts (except screening of household contacts) was not performed. Undetected carriage of MRSA of individuals in close proximity may have led to transmission and reinfection with MRSA by skin-to-skin contact and contact with contaminated objects [1]. Not all household contacts received eradication therapy simultaneously, which could also have contributed to reinfection of the index patient. Furthermore, eradication therapy was not always successful, as shown by prolonged carriage for months to years. The combination of long-term carriage and reinfection of index patients by individuals in their close proximity has probably contributed to the local spread of ST80 infections in relatively small areas and time span.

In conclusion, in the northern Netherlands, the high occurrence of PVL-positive MRSA infection and colonisation between 1998 and 2005 is mainly attributed to the ST80 strain. Ongoing transmission of ST80 in specific areas of the community may be explained by carriage of individuals passing colonisation and infection back and forth. Increasing occurrence of PVL-positive MRSA strains in the community will ultimately lead to migration of these strains to nursing homes and hospitals. Nursing homes may increasingly serve as a potential source of MRSA and an important risk factor for transmission to hospitals. Early MRSA detection, proper screening of patient contacts and adequate eradication therapy may in time help to contain further spread of PVL-positive MRSA strains in the community and, eventually, to health care environments.

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