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# The population structure of *Staphylococcus aureus* among general practice patients from The Netherlands

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## ABSTRACT

To investigate the prevalence, the antibiotic resistance pattern and the population structure of *Staphylococcus aureus*, *S. aureus* isolates from the anterior nostrils of patients of general practitioners (GPs) were analysed. Insight into the *S. aureus* population structure is essential, as nasal carriers of *S. aureus* are at increased risk of developing an *S. aureus* infection. *S. aureus* was isolated from nasal swabs from 2691 patients with no sign of an infection collected in 29 GP practices in The Netherlands. The susceptibility pattern for several classes of antibiotics was determined, as well as the *S. aureus* genetic background, using spa typing. *S. aureus* was isolated from 617 of the 2691 (23%) nasal swabs. The prevalences of resistance to ciprofloxacin, co-trimoxazole, fusidic acid, macrolides and mupirocin were 0.2%, 0%, 6%, 5% and 1%, respectively. Half of the isolates were associated with a genetic background common to the major methicillin-resistant *S. aureus* (MRSA) clones, e.g. clonal complex (CC)1, CC5, CC8, CC22, CC30 and CC45, and the remainder were mainly associated with CC7, CC12, CC15, CC26, CC51 and CC101. The low prevalences of resistance suggest that, in the Dutch situation, *S. aureus* isolates from patients visiting their GP because of complaints not related to infection do not represent a large reservoir of antibiotic resistance genes. Although no MRSA isolates were found, the genetic background of some of the *S. aureus* isolates is commonly observed among community-associated (CA)-MRSA clones (CC1, CC8 and CC30), and this might suggest that these isolates have the potential to become CA-MRSA.

## INTRODUCTION

*Staphylococcus aureus* is a potential hospital-associated (HA) and community-associated (CA) pathogen that can cause a wide variety of infectious diseases, ranging from minor skin infections to postoperative wound infections and necrotizing pneumonia [1]. It has been shown previously that nasal carriers of *S. aureus* are at increased risk of developing an *S. aureus* infection [2]. In addition, it has been shown that *S. aureus* of any genotype can become a life-threatening pathogen, but that some clones are more virulent than others [3].

Methicillin-resistant *S. aureus* (MRSA) is an increasing problem worldwide, in the form of both HA-MRSA and CA-MRSA. In The Netherlands, the prevalence of MRSA in hospitals has doubled during the last few years, from 1% in 2002 to 2% in 2006 [4]. Furthermore, several studies have reported the emergence of CA-MRSA in The Netherlands [5,6]. CA-MRSA can cause necrotizing pneumonia and severe skin infections in patients not at risk of MRSA acquisition. CA-MRSA is characterized by the presence of Pantone–Valentine leukocidin (PVL), the mobile resistance determinant staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V, and a continent-specific genetic background, i.e. clonal complex (CC)1, CC8, CC30 or CC80 in Europe [7].

It has previously been hypothesized that MRSA originated through the transfer of SCC*mec* from MRSA or methicillin-resistant coagulase-negative staphylococci to extant methicillin-susceptible *S. aureus* (MSSA) lineages, and that the genetic background of *S. aureus* determines the stability of the new MRSA clone [8,9]. However, the origin of CA-MRSA is not known, i.e. whether SCC*mec* has been acquired by MSSA in the community, or whether CA-MRSA is derived from HA-MRSA. Okuma et al. [10] showed that CA-MRSA represent novel acquisitions of SCC*mec* type IV in the community. However, Aires de Sousa et al. [11] raised the possibility that some CA-MRSA clones may originate in hospitals, as several similarities between CA-MRSA and HA-MRSA isolates were found.

In addition to the increased prevalence of MRSA, there is a growing concern regarding the prevalence of antibiotic-resistant microorganisms. Antibiotic use is generally considered to be the main risk factor for antibiotic resistance. Therefore, optimal use of antibiotics may contribute to the control of the problem of antibiotic resistance. Thus, for optimal use of antibiotics, i.e. the right empirical choice for the bacterial population, actual data on antibiotic resistance are essential. However, in The Netherlands, no actual data concerning antibiotic resistance of *S. aureus*, the main causative agent of skin infections, among general practitioner (GP) patients, are available.

The present study investigated the prevalence of antibiotic resistance of *S. aureus* isolates from patients visiting their GP who had no sign of an infection, with the aim of guiding antibiotic prescribing by GPs. Furthermore, the population structure of the *S. aureus* isolates was determined using *spa* typing to investigate whether the *S. aureus* isolates had a genetic background commonly observed among CA-MRSA isolates.

## MATERIALS AND METHODS

### Study population

Patients visiting 29 GP practices, distributed among four regions in The Netherlands, participated in this study. The majority of these practices participated in the sentinel project of The Netherlands Institute for Health Services Research. The number of participating practices per region was as follows: two from the northern part of The Netherlands (region I), three from the eastern part (region II), 11 from the middle (region III), and 13 from the southern part (region IV).

During 2005, from 2691 patients with no sign of an infection, a nose swab was taken from the anterior nostrils, including 186 from region I, 296 from region II, 901 from region III, and 1308 from region IV. Each participating GP included, at random, 50–100 patients (age >12 years). The *S. aureus* prevalence data and the antibiotic susceptibility patterns of

the *S. aureus* strains from his or her patients were sent to the GP anonymously. This study was approved by the ethical committee of the University Hospital Maastricht.

### Isolation of *S. aureus*

The nasal swabs were sent to the Department of Medical Microbiology of the University Hospital Maastricht for further analyses. The swabs were analysed for the presence of *S. aureus* using standard microbiological methods, which included culture on medium containing colistin and naladixic acid (BD Diagnostics, Erembodegem-Aalst, Belgium) and nutrient broth (Oxoid, Badhoevedorp, The Netherlands) containing 6.5% NaCl, as well as oxacillin resistance screening agar (Oxoid, The Netherlands), for the isolation of MRSA. All isolates were identified as *S. aureus* by Gram stain, and catalase and coagulase testing [12].

### Antimicrobial susceptibility testing

The susceptibility pattern of the *S. aureus* isolates was determined according to CLSI guidelines, using the microbroth dilution method with Mueller–Hinton II cation-adjusted broth (Becton Dickinson, Franklin Lakes, NJ, USA), an inoculum of  $5 \times 10^5$  CFU/mL, and overnight incubation at 37°C [13]. The microtitre plates for the determination of the MIC contained freeze-dried antibiotics (MCS Diagnostics, Swalmen, The Netherlands). Susceptibility to the following antimicrobial agents (range in mg/L) was determined: cefaclor (0.06–128), cefuroxime (0.06–128), clindamycin (0.03–64), ciprofloxacin (0.25–4), clarithromycin (0.03–64), gentamicin (0.06–64), imipenem (0.03–64), linezolid (0.03–64), moxifloxacin (0.12–4), oxacillin (0.03–64), penicillin (0.004–8), rifampin (0.008–16), teicoplanin (0.06–128), tetracycline (0.03–64), trimethoprim–sulphamethoxazole (0.015/0.29–32/680) and vancomycin (0.06–128). Susceptibility to fusidic acid (100 µg) and mupirocin (10 µg) (Rosco, Taastrup, Denmark) was determined using the disk diffusion method according to CLSI guidelines [13]. All isolates resistant to clarithromycin were tested for inducible clindamycin resistance using the D-test according to CLSI guidelines [14].

### Genotypic determinations

The oxacillin-resistant isolates were analysed for the presence of the *mecA* gene, using a real-time PCR assay that was developed in the Department of Medical Microbiology of the University Hospital Maastricht. The primers and the TaqMan probe for the detection of *mecA* were designed on the basis of the published sequence (GenBank accession no. X52593) of the *mecA* gene [15]. The sequences of the forward (*mecA*\_FP) and reverse (*mecA*\_RP) primer were 5'-TGAAGTGGTAAATGGTAATATCGACTTAA-3' and 5'-TAATTTCGAGTGCTACTCTAGCAAAGAA-3', respectively (Sigma Genosys, Haverhill, UK). The sequence of the VIC-labeled MGB probe (*mecA*\_PR) was 5'-CAAGCAATAGAATCATCAGATAA-3' (Applied Biosystems, Nieuwerk a/d IJssel, The Netherlands). A real-time PCR for the *S. aureus*-specific *femA* gene served as an internal control [16]. The following reaction conditions were used in the TaqMan assay: 0.3 µM *femA*\_FP, 0.3 µM *femA*\_RP, 100 nM *femA*-PR, 0.6 µM *mecA*\_FP, 0.6 µM *mecA*\_RP, 125 mM *mecA*\_PR, 1 × TaqMan Universal PCR Master Mix and 4 µL of a 0.5 McFarland suspension ( $1.5 \times 10^8$  CFU/mL) in a total volume of reaction mixture of 50 µL. Amplification was performed on the ABI PRISM 7000 Sequence Detection System, using the following programme: 2 min at 50°C and 10 min at 95°C, followed by 42 cycles of 15 s at 95°C and 60 s at 60°C.

The presence of the *llecS-2* gene, coding for high-level mupirocin resistance, was investigated by PCR as described previously [17].

Real-time amplification of the *spa* locus, followed by sequencing, was performed as described previously [18]. The *spa* types were clustered into *spa*-CCs using the algorithm based upon repeat pattern (BURP) with the Ridom StaphType version 1.5 software package (<http://www.ridom.de>). The default settings recommended by the manufacturer were used. As it has been shown that *spa* typing, together with the algorithm BURP, yields results that

are in concordance with typing results obtained by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis [18,19], the associated CCs, as determined with MLST, were allocated through the Ridom SpaServer (<http://spaserver.ridom.de>).

The presence of PVL was investigated on a random subset of 100 isolates with a diverse genetic background, using real-time PCR [20].

### Statistical analyses

Significant statistical differences were calculated using the Mann–Whitney U-test, using SPSS 14.0 (SPSS Inc., Gorinchem, The Netherlands). A p-value of <0.05 was considered to be statistically significant.

## RESULTS

### Antibiotic susceptibility pattern

*S. aureus* was isolated from 617 of the 2691 nose swabs received, resulting in a prevalence of 23%. Only one sample from each patient was included in the study. Of the 617 isolates, 595 were available for further analyses; the remaining 22 isolates could not be cultured from the frozen stocks. All isolates were sensitive to clindamycin, trimethoprim–sulphamethoxazole and vancomycin. Penicillin resistance was observed among 68% of the isolates. Resistance to oxacillin was observed in four isolates. The MIC values were 4 and 8 mg/L for two strains each. None of the 595 *S. aureus* strains harboured the *mecA* gene, and they were therefore not classified as MRSA. Resistance to clarithromycin and susceptibility to clindamycin was observed in 30 isolates (5%). The D-test showed that 17 of these *S. aureus* isolates (57%) had the inducible clindamycin resistance phenotype. Resistance to fusidic acid was observed in 36 isolates (6%), and 2% of the isolates (n = 20) were resistant to tetracycline. Resistance to mupirocin was observed in two isolates, which harboured the *lIeS-2* gene (Table 1).

### [TABLE 1]

No statistically significant differences (p >0.05) were observed in the distribution of the resistance patterns in the four regions.

### Distribution of spa types and BURP analyses

The 595 MSSA isolates had 244 different spa types, and BURP analysis classified these into 23 spa-CCs (including seven with no founder) and 46 singletons. Twenty-five spa types were excluded from the analysis because the spa region was less than five spa repeats in length (Table 2). New spa types were observed among 48 of the 244 spa types (20%). The most common spa types observed were t002 (4.1%), t005 (1.7%), t008 (4.1%), t012 (5.6%), t015 (2.9%), t021 (3.4%), t026 (2.5%), t056 (1.7%), t084 (3.6%), t091 (5.6%) and t127 (3.6%). Each of the remaining 233 spa types (61.2% of the isolates) accounted for between 0.2% and 1.4% each.

### [TABLE 2]

The most common spa-CCs were spa-CC 012 (15%), spa-CC 015 (11%), spa-CC 002 (7%), spa-CC 024 (6%), spa-CC 127 (5%), spa-CC 005 (3%), spa-CC 122 (2%) and spa-CC 216 (2%). These spa-CCs were associated with genetic backgrounds commonly found among endemic MRSA clones, i.e. CC30, CC45, CC5, CC8, CC1, CC22, CC30 and CC59, respectively (Table 2). Further major spa-CCs were spa-CC 084 (14%), spa-CC 078 (5%), spa-CC 166 (4%) and spa-CC 645/159 (4%). These spa-CCs are associated with CC7/15, CC26/101, an unknown CC, and CC51 respectively, usually observed among MSSA clones. The remaining spa-CCs accounted for <1% of the MSSA isolates (Table 2). The four borderline oxacillin-resistant *S. aureus* (BORSA) isolates were associated with MLST CC1, CC5, CC12 and CC97.

No statistically significant differences ( $p > 0.05$ ) were observed in the distribution of the *spa*-CCs in the four regions (Table 3).

### [TABLE 3]

#### Prevalence of PVL

None of the MSSA isolates tested harboured PVL.

#### DISCUSSION

The prevalence of nasal colonization with *S. aureus* in the Dutch community (23%) was in agreement with the prevalence in patients admitted to Dutch hospitals (24.4%) [21]. The results of the present study showed a low prevalence of resistance among *S. aureus* isolates cultured from the nose of patients attending their GP with no sign of an infection. The highest resistance rate was found for penicillin, i.e. 68%, which is still relatively low as compared to the resistance levels found among clinical isolates [1]. The low prevalence of resistance to the antibiotics commonly used by GPs is in line with the general observation of low extramural antibiotic use in The Netherlands [22]. The higher resistance to fusidic acid in comparison with mupirocin (6% vs. <1%) reflects the higher use of fusidic acid than of mupirocin in cases of proven or possible staphylococcal infection. The latter compound is indicated for use only in cases of MRSA colonization. The low rates of resistance to mupirocin and fusidic acid support the current Dutch GP standard for treatment of skin infections. Fusidic acid is the antibiotic of first choice for skin infections, whereas mupirocin is indicated for *S. aureus* infections caused by fusidic-resistant *S. aureus* strains. However, the population structure of nasal carriage isolates and clinical isolates can be different [3]. It has been shown that resistance genes can be located on mobile genetic elements in the genome of *S. aureus*, e.g. transposon Tn554 carrying the *ermA* gene. In addition, *S. aureus* strains can carry plasmids on which resistance genes are present. Both transposons and plasmids are mobile and can thus be transferred to other *S. aureus* strains of different lineages, possibly due to antibiotic pressure [23].

The four isolates for which the MIC of oxacillin was 4 or 8 mg/L, but which did not harbour the *mecA* gene, were classified as BORSA [24]. Isolates of this kind have been described previously in a few reports, mostly in a clinical setting, and the mechanism of  $\beta$ -lactam resistance is not clear at the moment, but could involve increased production of  $\beta$ -lactamases [24], or several amino acid substitutions in penicillin-binding protein 2 [25]. The presence of BORSA as part of the commensal nasal flora has, to the best of our knowledge, not been described previously. The clinical significance for the individual patient, or for the patient population in general, has not been investigated, and it is not known whether these isolates are 'precursors' for MRSA and/or whether these isolates spread as easily as MRSA. Although the mechanism of resistance of BORSA and that of MRSA are different, the four BORSA strains had genetic backgrounds (CC1, CC5, CC12 and CC97) that have previously been observed among MRSA isolates. CC1 and CC5 are endemic MRSA lineages [26], whereas sporadic MRSA isolates associated with CC12 and CC97, i.e. ST12-MRSA-IV and ST97-MRSA-IV, have been observed before [27,28]. Furthermore, it has been suggested previously that low-level resistance could be the gateway to high-level resistance [29].

For this study, *spa* typing, together with BURP analyses, was used to determine the genetic background of the *S. aureus* isolates. Next, the *spa* typing/BURP data were associated with the MLST CC on the SpaServer, as has been done previously [30–33].

Several *spa* types correspond to a single sequence type (ST) as determined with MLST, but they remain within an assigned clonal cluster. Furthermore, several studies have shown a good correlation between MLST and *spa* typing/BURP [18,19]. A disadvantage of *spa* typing is that it sometimes lacks discriminatory power, due to the related *spa* repeat patterns within different clonal lineages, possibly caused by recombination events involving the *spa*

locus [18]. However, careful study of the figures generated by BURP analyses can resolve this problem [30].

In the present study, 52% of the MSSA isolates that could be classified into *spa*-CCs had a genetic background commonly observed in either epidemic HA-MRSA clones, i.e. CC5, CC8, CC22, CC30 and CC45 [26], or CA-MRSA clones, i.e. CC1, CC8, CC30 and CC59 [7]. This percentage is comparable to that found in a recent study in Belgium, in which 45% of the MSSA isolates had a genetic background common to the major MRSA clones [34]. MSSA isolates with a CC1, CC5, CC8, CC30 and CC45 background have been described previously in Brazil and Germany, in Danish isolates from the 1960s and 1970s, and in the Dutch and English community [35–40]. CC59 is a common genetic background among CA-MRSA isolates in Asian countries, e.g. Singapore and Taiwan, but has also been observed recently in The Netherlands [7,41–43]. A recent study among children and elderly people in The Netherlands showed that CC30 and CC45 were the most prevalent (47.3%) MSSA clones in the Rotterdam area in the west of The Netherlands [44]. The present study found these MSSA clones in only 26% of the study population. A reason for the difference in prevalence could be the population studied, or the fact that the present study covered GP patients from throughout The Netherlands. Although an outbreak of the community-associated ST80-MRSA-IV clone has recently been described in the north of The Netherlands, no MSSA isolates with this genetic background were observed in the present study [6]. The fact that MSSA isolates with a genetic background common to CA-MRSA clones were found might suggest that these MSSA isolates could be recipients for SCCmec type IV or V, as these elements are suggested to be highly mobile [8], as has been shown previously with the *S. aureus* CC30 clone. This clone was prevalent in the 1950s as a penicillin-resistant *S. aureus* clone, but is now re-emerging, both in the hospital environment as the ST36-MRSA-II clone, and in the community as the PVL-positive ST30-MRSA-IV clone [26,45]. The transfer of SCCmec has been shown to occur frequently in the global evolution of MRSA [39,46,47]. However, high-resolution typing using MLST and *S. aureus* surface (*sas*) genes is needed to investigate the possible transfer of SCCmec into these MSSA lineages [39].

Several *S. aureus* clones with a genetic background that differs from the major MRSA clones, e.g. CC7, CC12, CC15, CC26, CC51, CC97 and CC101, were found. The observation that more MSSA CCs were found than CCs from endemic MRSA clones suggests that the MSSA population is more heterogeneous. Similar observations have been made in Belgium, Brazil, Germany and Portugal [34,37,48,49]. Recently, MSSA isolates of CC7, CC9, CC12, CC15, CC25, CC51 and CC101 have been observed in Belgium and in the English community, although no MRSA isolates with these genetic backgrounds were found [34,40]. Similarly, in Portugal, MSSA isolates of CC9, CC12, CC15, CC25 and CC51 have been found in the community and the hospital environment, whereas no MRSA isolates grouped into these CCs were observed [48]. The CC51 genetic background has been found among Danish MSSA isolates from the 1960s, and among Dutch MSSA isolates observed between 1997 and 2002 in the community [3,36]. These observations support the existence of successful MSSA lineages, e.g. CC7 and CC15, both in the Dutch community and beyond. This also suggests that these MSSA lineages, as compared to other lineages, possess characteristics that favour their persistence in the host, as well as transfer between hosts. Further research is necessary to investigate these characteristics.

The absence of PVL-positive isolates is in agreement with the study of Melles et al., which revealed a low PVL prevalence among isolates from nasal carriers (0.6%) and among blood culture isolates (2.1%). However, a PVL prevalence of 38.9% was observed among *S. aureus* strains that caused abscesses and arthritis, which is in agreement with the involvement of PVL in soft tissue infections [3].

In conclusion, the results suggest that the *S. aureus* isolates tested did not comprise a large reservoir of antibiotic resistance genes. As the MSSA isolates observed had a heterogeneous

genetic background, both common and uncommon to major MRSA clones, and as SCCmec type IV and V are mobile, it is likely that new CA-MRSA clones could emerge in the future.

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G. A. Donker and R. H. Deurenberg contributed equally to this study. The authors thank all the GPs who participated in the study.

#### **TRANSPARENCY DECLARATION**

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**TABLES**

Table 1. Antibiotic susceptibility pattern by region

| Region | Total     | No. (%) resistant <i>S. aureus</i> isolates |          |       |       |       |        |        |       |       |        |
|--------|-----------|---|----------|-------|-------|-------|--------|--------|-------|-------|--------|
|        |           | CIP   | PEN      | OXA   | GEN   | FAC   | CLA    | TET    | RIF   | MUP   | FUC    |
| I      | 49 (8)    | 1 (2)                                       | 32 (65)  | 0 (0) | 0 (0) | 0 (0) | 2 (4)  | 2 (4)  | 0 (0) | 1 (2) | 4 (8)  |
| II     | 50 (8)    | 0 (0)                                       | 35 (70)  | 1 (2) | 0 (0) | 0 (0) | 1 (2)  | 2 (4)  | 1 (2) | 0 (0) | 4 (8)  |
| III    | 211 (36)  | 0 (0)                                       | 147 (70) | 1 (1) | 1 (1) | 0 (0) | 11 (5) | 9 (4)  | 0 (0) | 0 (0) | 11 (5) |
| IV     | 285 (48)  | 2 (1)                                       | 190 (67) | 2 (1) | 1 (0) | 2 (1) | 16 (6) | 7 (2)  | 0 (0) | 1 (0) | 17 (6) |
| Total  | 595 (100) | 3 (1)                                       | 404 (68) | 4 (1) | 2 (0) | 2 (0) | 30 (5) | 20 (3) | 1 (0) | 2 (0) | 36 (6) |

All isolates were sensitive to cefuroxime, clindamycin, imipenem, linezolid, moxifloxacin, teicoplanin, trimethoprim–sulphamethoxazole, and vancomycin.

Table 2. Composition of the spa-CCs

| <i>spa</i> -CC         | No. (%) of isolates | No. (%) of <i>spa</i> types | <i>spa</i> types <sup>a</sup>   | Associated CC <sup>b</sup> |
|------------------------|---------------------|-----------------------------|---|----------------------------|
| <i>spa</i> -CC 015     | 67 (11)             | 27 (11)                     | <u>t505, t583, t589, t620, t630, t772, t908, t950, t1574, t2135, t2239, t2254, t2539, t2541, t2544, t2568, t2682</u>  | 45                         |
| <i>spa</i> -CC 012     | 89 (15)             | 26 (11)                     | <u>t404, t406, t483, t822, t840, t1130, t1239, t1504, t1932, t2209, t2210, t2489, t2561, t2566, t2572, t2821</u>  | 30                         |
| <i>spa</i> -CC 084     | 81 (14)             | 16 (7)                      | <u>t084, t085, t091, t279, t346, t360, t491, t774, t853, t867, t1363, t1716, t2074, t2543, t2567, t2616</u>   | 7/15                       |
| <i>spa</i> -CC 078     | 27 (5)              | 14 (6)                      | <u>t056, t078, t081, t087, t150, t258, t353, t775, t1102, t1312, t1541, t1671, t1898, t2039</u>   | 26/101                     |
| <i>spa</i> -CC 166     | 21 (4)              | 11 (5)                      | <u>t089, t136, t153, t166, t240, t369, t1014, t2038, t2071, t2073, t2854</u>  |                            |
| <i>spa</i> -CC 645/159 | 15 (3)              | 9 (4)                       | <u>t159, t171, t272, t284, t408, t645, t659, t738, t2213</u>  | 51                         |
| <i>spa</i> -CC 005     | 18 (3)              | 9 (4)                       | <u>t005, t060, t223, t474, t790, t1433, t1629, t2618, t2681</u>   | 22                         |
| <i>spa</i> -CC 002     | 40 (7)              | 9 (4)                       | <u>t002, t010, t179, t242, t306, t311, t447, t2212, t2491</u>   | 5                          |
| <i>spa</i> -CC 024     | 38 (6)              | 9 (4)                       | <u>t008, t024, t190, t648, t701, t711, t846, t1171, t2041</u>   | 8                          |
| <i>spa</i> -CC 122     | 13 (2)              | 7 (3)                       | <u>t019, t122, t138, t2387, t2496, t2540, t2610</u>   | 30                         |
| <i>spa</i> -CC 127     | 28 (5)              | 5 (2)                       | <u>t127, t177, t591, t1787, t2500</u>   | 1                          |
| <i>spa</i> -CC 216     | 11 (2)              | 4 (2)                       | <u>t172, t216, t2079, t2488</u>   | 59                         |
| <i>spa</i> -CC 189     | 5 (1)               | 4 (2)                       | <u>t189, t2569, t2612, t2819</u>  | 1                          |
| <i>spa</i> -CC 359     | 4 (1)               | 3 (1)                       | <u>t224, t359, t1236</u>  | 97                         |
| <i>spa</i> -CC 160     | 7 (1)               | 3 (1)                       | <u>t160, t213, t771</u>   | 12                         |
| <i>spa</i> -CC 1149    | 4 (1)               | 3 (1)                       | <u>t937, t1149, t2077</u>   |                            |
| No founder 17          | 4 (1)               | 2 (1)                       | <u>t156, t1702</u>  | 12                         |
| No founder 18          | 3 (1)               | 2 (1)                       | <u>t246, t2495</u>  |                            |
| No founder 19          | 2 (0)               | 2 (1)                       | <u>t034, t571</u>   |                            |
| No founder 20          | 3 (1)               | 2 (1)                       | <u>t148, t2016</u>  |                            |
| No founder 21          | 2 (0)               | 2 (1)                       | <u>t186, t729</u>   | 88                         |
| No founder 22          | 2 (0)               | 2 (1)                       | <u>t814, t2078</u>  |                            |
| No founder 23          | 6 (1)               | 2 (1)                       | <u>t364, t493</u>   |                            |
| Singletons             | 56 (9)              | 46 (19)                     | <u>t062, t099, t106, t164, t209, t252, t276, t286, t334, t344, t370, t377, t389, t469, t587, t631, t878, t884, t1045, t1362, t1406, t1943, t2050, t2070, t2075, t2076, t2080, t2208, t2479, t2490, t2492, t2494, t2542, t2547, t2548, t2556, t2557, t2558, t2559, t2570, t2573, t2615, t2617, t2674, t2680, t2820</u> | 5/30                       |
| Excluded <sup>c</sup>  | 44 (7)              | 25 (10)                     | <u>t026, t059, t233, t287, t362, t386, t502, t524, t535, t643, t808, t1152, t1200, t1209, t1456, t2176, t2207, t2211, t2246, t2383, t2493, t2571, t2611, t2613, t2614</u>   | 45                         |
| Non-typeable           | 4 (1)               |                             |   |                            |
| Novel repeat           | 1 (0)               |                             |   |                            |
| Total                  | 595 (100)           | 244                         |   |                            |

<sup>a</sup>New *spa* types are underlined.

<sup>b</sup>CC, clonal complex as determined with multilocus sequence typing.

<sup>c</sup>*spa* types smaller than five *spa* repeats.

TABLE 3. Distribution of the *spa*-CCs by region

| <i>spa</i> -CC         | No. of isolates in region |    |     |     |
|------------------------|---------------------------|----|-----|-----|
|                        | I                         | II | III | IV  |
| <i>spa</i> -CC 015     | 5                         | 4  | 28  | 30  |
| <i>spa</i> -CC 012     | 7                         | 13 | 26  | 43  |
| <i>spa</i> -CC 084     | 6                         | 5  | 29  | 41  |
| <i>spa</i> -CC 078     | 1                         | 3  | 4   | 19  |
| <i>spa</i> -CC 166     | 0                         | 2  | 11  | 8   |
| <i>spa</i> -CC 645/159 | 1                         | 1  | 7   | 6   |
| <i>spa</i> -CC 005     | 2                         | 3  | 8   | 5   |
| <i>spa</i> -CC 002     | 3                         | 1  | 17  | 19  |
| <i>spa</i> -CC 024     | 5                         | 2  | 15  | 16  |
| <i>spa</i> -CC 122     | 1                         | 0  | 5   | 7   |
| <i>spa</i> -CC 127     | 0                         | 2  | 11  | 15  |
| <i>spa</i> -CC 216     | 2                         | 1  | 1   | 7   |
| <i>spa</i> -CC 189     | 0                         | 1  | 3   | 1   |
| <i>spa</i> -CC 359     | 1                         | 1  | 1   | 1   |
| <i>spa</i> -CC 160     | 0                         | 0  | 5   | 2   |
| <i>spa</i> -CC 1149    | 1                         | 0  | 1   | 2   |
| No founder 17          | 0                         | 0  | 0   | 4   |
| No founder 18          | 0                         | 1  | 0   | 2   |
| No founder 19          | 0                         | 0  | 1   | 1   |
| No founder 20          | 0                         | 0  | 3   | 0   |
| No founder 21          | 0                         | 0  | 1   | 1   |
| No founder 22          | 0                         | 0  | 2   | 0   |
| No founder 23          | 0                         | 1  | 1   | 4   |
| Singletons             | 6                         | 5  | 18  | 27  |
| Excluded <sup>a</sup>  | 7                         | 4  | 11  | 22  |
| Non-typeable           | 0                         | 0  | 2   | 2   |
| Novel repeat           | 1                         | 0  | 0   | 0   |
| Total                  | 49                        | 50 | 211 | 285 |

CC, clonal complex.  
<sup>a</sup>*spa* types smaller than five *spa* repeats.