Changes in staphylococcal cassette chromosome type and antibiotic resistance profile in methicillin-resistant *Staphylococcus aureus* isolates from a French hospital over an 11 year period

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**Background:** In this study, we investigated the relationship between changes in antibiotic resistance and distribution of staphylococcal cassette chromosome (SCC) types amongst methicillin-resistant *Staphylococcus aureus* (MRSA) isolates expressing the most frequently encountered profiles of antibiotic resistance over an 11 year period in the University Hospital of Rennes, France.

**Methods:** Antibiotic susceptibilities were determined by agar diffusion. SCC typing was performed using PCR. PFGE demonstrated that isolates were phylogenetically related.

**Results:** Fourteen profiles of antibiotic resistance were defined among MRSA isolates. For each resistance profile, only one SCC type was associated: four patterns corresponded to SCC type I or IA, nine to SCC type IV or IVA, and none to types II and III. One was not typed. PFGE indicated that isolates with SCC type I or IA belong to a single genetic lineage, which also includes most of the epidemic isolates, which carry SCC type IVA. In contrast to type I or IA, isolates with SCC type IV or IVA were found to be associated with several different PFGE clusters, although not all of these represent epidemic isolates.

**Conclusions:** During the course of the study, the spectrum of antibiotic resistance in MRSA isolates decreased. This occurred due to the emergence of strains with SCC type IV or IVA, which are susceptible to more antibiotics than type I or IA strains. The greater prevalence of such isolates could not be linked conclusively to the presence of SCC type IV or IVA, or to one particular PFGE cluster.

**Keywords:** *S. aureus*, MRSA, mecA, SCC, resistance profiles

**Introduction**

Since the first identification of a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate in 1960 in the UK, organisms with this phenotype have been found throughout the world, and are responsible for nosocomial infections associated with higher mortality than infections due to methicillin-susceptible *S. aureus* (MSSA) isolates.¹

Recently, important advances in the study of MRSA phylogenetics, based on the characterization of the sequence of the methicillin-resistance gene, mecA, and of the genetic elements that carry this gene in different organisms, have provided evidence that methicillin resistance has evolved in different genetic lineages of MSSAs by means of horizontal transfer of various staphylococcal chromosome cassettes (SCC).²-⁶ SCCs are mobile elements characterized by association of a mec complex and ccr genes coding for integration into or excision from the chromosome.⁷ Three types of SCC (types I, II and III) were originally described in hospital-acquired MRSA strains (HA-MRSA), most of them isolated before 1990.⁶-⁸ A fourth type (type IV) was recently described, first in community-acquired MRSA isolates (CA-MRSA) and then in several MRSA backgrounds, including hospital isolates.⁸-¹⁰

In the Centre Hospitalier Universitaire, Rennes, France, surveillance of overall antibiotic resistance in MRSA isolates has demonstrated a significant change during the 1990s: gentamicin-resistant, multi-resistant strains have almost disappeared since 1997 and have been replaced by gentamicin-susceptible, less resistant MRSA isolates.¹¹ Gentamicin-susceptible MRSA strains have been epidemic in most hospitals in France since 1992.¹²-¹⁴ The aims of this study were to type representative isolates expressing the most frequently
encountered MRSA resistance profiles seen during 1992–2002 and to describe how these resistance profiles have changed over time. Furthermore, we set out to determine whether there is a link between resistance profile and the SCC types carried by these MRSA isolates.

Materials and methods

Bacterial strains

The University Hospital of Rennes is a 1978 bed teaching hospital. For the period 1992–2002, inclusive, the mean annual incidence of methicillin resistance among S. aureus isolates was 36% ± 2%. During this period, 20,619 S. aureus isolates were recovered from samples from patients hospitalized in four of five facilities. Isolates were identified as S. aureus by their ability to produce acid on Chapman agar, and by their production of catalase and coagulase.

Changes in antibiotic resistance profiles among MRSA isolates

Antimicrobial susceptibility was measured using the agar diffusion method (Bio-Rad, Marnes-la-Coquette, France) on Mueller–Hinton agar (Oxoid, Dardilly, France) according to the recommendations of the Anti-

biogram Committee of the French Microbiology Society (CA-SFM), except that isolates with a zone inhibition diameter for fosfomycin >14 mm and <23 mm were categorized as intermediately resistant to this antibiotic. Patient information (sex, age, clinical specimen) and antibiotic results were collected from the laboratory information system and stored in a specific database. If several isolates with the same anti-

biogram were recovered at different times from one patient, only the first isolate was retained. In total, 4,080 MRSA isolates were retained for further analysis. Temporal changes in antibiotic resistance profiles among these MRSA isolates were studied by use of the EPILOG software (Medasys, Gif-sur-Yvette, France).

Definition of resistance profiles

MRSA isolates were grouped by overall resistance profile, as reported previously. A resistance profile was characterized by a combination of results (S, susceptible and R, intermediate or resistant) for the following antibiotics: neomycin, tobramycin, gentamicin, erythromycin, linco-

mycin, sulfamethoxazole, pefloxacine, rifampicin and fosfomycin. An identical resistance profile, found in 10 or more MRSA during the same year, was considered as a ‘frequent’ MRSA resistance profile in that year.

PFGE

For MRSA isolates before 1998, PFGE was performed with a Gene Navigator apparatus (Amersham Pharmacia, Orsay, France), and for strains isolated after 1998, PFGE was performed with a CHEF-II system (Bio-Rad, Ivry-sur-Seine, France). Gel Compar software (Applied Maths, Sint-Martens-Latem, Belgium) was used to calculate Dice similarity indices and to perform cluster analysis by un-weighted pair group matching indices (tolerance 2.0%). Two isolates were considered as genetically related if their Dice similarity index was equal to 85% or more.

Typing of staphylococcal chromosome cassette

Two, three or four isolates expressing each MRSA resistance profile were selected according to year of isolation. Since isolates with resistance profile V were no longer available, this profile has been excluded from further study. Bacterial DNA was extracted using a QiAamp tissue kit from Qiagen (Courtaboeuf, France). SCC typing was performed with a multiplex-PCR method according to Oliveira & de Lencastre.

Results

Changes in antibiotic resistance amongst MRSA isolates are described in Figure 1. During 1995–1998, the number of MRSA isolates expressing resistance to one or more of gentamicin, minocy-

cline, rifampicin or sulfamethoxazole decreased dramatically. The number of lincomycin-resistant MRSA isolates first decreased over the period 1992–1995 and subsequently increased. Slight variations were also observed for the proportion of MRSA isolates resistant to erythromycin at the beginning and at the end of the study. In contrast, no change was observed in the proportion of isolates that were resistant to one or more of fusidic acid, tobramycin or fluoroquinolones.

By use of EPILOG software, 14 frequent MRSA resistance profiles (i.e. groups of isolates that express resistance to the same anti-

biotics) could be defined (Table 1). Variations in the isolation rate of the six most frequent profiles during the course of the study are reported in Figure 2.

PFGE data, to show overall relatedness amongst the isolates with different resistance profiles, were available from laboratory data-

bases. However, the method used to perform PFGE changed in 1998. Accordingly, it is not possible to assess the overall relatedness of isolates collected in 1990–1997 with those collected in 1998–2002. Consequently, comparisons of the relationship between antibiotic resistance profiles and PFGE profiles were performed in two separate groups, one for isolates collected during 1990–1997 and the other for isolates collected during 1998–2002. Isolates with resistance profile VII (Table 1) were of particular interest, and so PFGE data were collected for all of them using both PFGE methods. The PFGE data are shown in Figure 3. MRSA strains isolated before 1998 were separated in two PFGE clusters, N1 and N2. These have 62% related-

ness according to the software used. PFGE cluster N1 (deviation of relatedness = 11%) included all MRSA isolates with antibiotic resistance profiles I (n = 4), IV (n = 2), VI (n = 3) and VII. PFGE cluster N2 (deviation of relatedness = 7%) contains MRSA isolates with resistance patterns II (n = 2) and III (n = 3). For MRSA isolates collected since 1998, all isolates with resistance profiles VI (n = 1), VII (n = 4), VIII (n = 3), XIII (n = 3) and XIV (n = 3) were present in PFGE cluster C1, and those with resistance profile IX (n = 3) were present in PFGE

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Table 1. Characteristics and typing results of MRSA isolates expressing different resistance profiles

<table>
<thead>
<tr>
<th>Profile</th>
<th>Year(s) of maximal isolation rate(s)</th>
<th>No. of infected patients (1992–2002)</th>
<th>Spectrum of resistance</th>
<th>PFGE and SCC typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1992</td>
<td>138</td>
<td>S R R R S R R R R R</td>
<td>4 1992 N1 I</td>
</tr>
<tr>
<td>III</td>
<td>1993, 1994, 1995</td>
<td>417</td>
<td>R R R R R S R S S S</td>
<td>3 1994 N2 IVA</td>
</tr>
<tr>
<td>IV</td>
<td>1992, 1993</td>
<td>135</td>
<td>R R R R R R R S S S</td>
<td>2 1993 N1 IA</td>
</tr>
<tr>
<td>V</td>
<td>1993, 1994</td>
<td>35</td>
<td>R R R R R R R S S S</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>VI</td>
<td>1996, 1997</td>
<td>309</td>
<td>R R R R R R R S S S</td>
<td>4 1995, 1996, N1, C1 IA</td>
</tr>
<tr>
<td>VIII</td>
<td>1996, 2002</td>
<td>132</td>
<td>R R S R R R S S S S</td>
<td>3 1999 C1 IVA</td>
</tr>
<tr>
<td>X</td>
<td>1999, 2001</td>
<td>55</td>
<td>S S S S S S S S S S</td>
<td>3 1999, 2001 C1, C2, SI IV</td>
</tr>
<tr>
<td>XIII</td>
<td>2000, 2001</td>
<td>49</td>
<td>R R R R R R R R R R</td>
<td>3 2000, 2001 C1 IA</td>
</tr>
</tbody>
</table>

NEO, neomycin; TOB, tobramycin; GEN, gentamicin; ERY, erythromycin; LIN, lincomycin; SUL, sulfamethoxazole; PEF, pefloxacin; RIF, rifampicin; FOF, fosfomycin; R, resistant or intermediate; S, susceptible.

*N1 and N2, clusters from analysis of PFGE using the Gene Navigator system; C1 and C2, clusters from analysis of PFGE using the CHEF-II system; SI, single isolates (not related to a cluster) from analysis of PFGE using the CHEF-II system.
SCC types and antibiotic resistance profiles in MRSA isolates

Cluster C2. MRSA isolates with resistance profiles X \((n = 3)\), XI \((n = 3)\) and XII \((n = 3)\) were not grouped into a single PFGE cluster.

SCC typing of representative isolates from each antibiotic-resistance profile was performed, except for isolates with profile V. Results of SCC typing were unambiguous: for one resistance pattern, only one SCC type was found among isolates tested. The characteristics and SCC type of each resistance pattern are shown in Table 1: four are SCC type I or IA and nine are SCC type IV or IVA.

Discussion

Advances in the phylogenetic study of MRSA isolates have been made by studying variations among SCC clusters, and through sequencing housekeeping genes. Results indicate that some genetic lineages of *S. aureus* have spread widely within hospitals after acquisition of *meca*. A recent re-analysis of MRSA epidemiology was stimulated after the discovery of SCC type IV in MRSA isolates from infections not related to hospitals. Such CA-MRSA isolates tend to be more susceptible to other antibiotics than are HA-MRSA, and their narrow spectrum of resistance is solely due to determinants harboured on genetic elements present on the SCC: for example, *Tn*554, which codes for resistance to spectinomycin and macrolides, or pUB110, which carries resistance to kanamycin and tobramycin. In our hospital, in contrast with HA-MRSA isolated in the early to mid 1990s, which tended to be multi-resistant, more recently encountered HA-MRSA have much greater levels of antibiotic susceptibility. Accordingly, in this study we set out to determine whether the general increase in antibiotic susceptibility seen in our hospital is associated with an increased prevalence of SCC type IV, typically seen in more susceptible MRSA isolates.

The data presented here show that since 1995 major changes (i.e. typically a decrease in the spectrum of resistance) observed in the antibiotic resistance of MRSA isolates have been directly linked to the emergence of SCC type IV or IVA. SCC type IV is a shorter and more mobile genetic element than other SCC types and it has been found to be associated with a wide variety of phylogenetic backgrounds of *S. aureus*. Since 1992, SCC type IVA has been the type most frequently observed among MRSA isolates in our hospital. It is found not only in gentamicin-susceptible and tobramycin-resistant MRSA but also in two profiles of resistance (II and III) that include gentamicin resistance and were frequently isolated before 1997. Because of this observation, it is unlikely that the gentamicin-resistance determinant is encoded on the SCC IV element. Accordingly, these data imply transfer of SCC IVA into the gentamicin-resistant MRSA background. The finding of SCC IVA in isolates with varying degrees of prevalence (e.g. isolates expressing resistance profiles VII and XII) indicates that acquisition of SCC IVA does not itself confer a particular advantage in causing epidemics. Nonetheless these strains seem widely disseminated in hospitals in Europe; large outbreaks due to EMRSA-15, an SCC type IV strain, have been reported in England, Wales and Germany.

In our study, SCC type I isolates were found to correspond to antibiotic resistance profile I, which was predominant during 1988–1991. SCC type I is specific for the Archaic clone. Archaic and Iberian MRSA clones are both characterized by SCC type I, but the Iberian SCC type (called IA) differs by the presence of a linearized pUB110 plasmid, which codes for the nucleotidyltransferase enzyme ANT4, inactivating kanamycin, neomycin, amikacin and tobramycin. This plasmid is also present in the SCC type IVA but not in type IV. This implies that resistance profiles corresponding to SCC types IA and IVA include resistance to neomycin, and those corresponding to SCC types I and IV do not, which has been confirmed in this study (Table 1). Consequently, a neomycin-susceptible MRSA isolate should be expected to have an SCC type I if it is resistant to gentamicin and an SCC type IV if it is susceptible to this drug. Other than neomycin and gentamicin, no other antibiotic or combination of antibiotics can

![Figure 2](https://academic.oup.com/jac/article-abstract/53/5/808/747197 by guest on 01 December 2018)  
Figure 2. Changes in the prevalence of isolates with selected antibiotic resistance profiles over time.

![Figure 3](https://academic.oup.com/jac/article-abstract/53/5/808/747197 by guest on 01 December 2018)  
Figure 3. Comparison of MRSA strains by use of pulsed-field gel electrophoresis.
be used to predict the SCC type carried by an isolate. Nonetheless, in this study, we have confirmed—as general rule—that isolates carrying SCC type IV or IVA are susceptible to more antibiotics than others (three antibiotics at least), as described previously for HA-MRSA isolates related to the Paediatric clone in Lisbon, HA-MRSA in German hospitals or CA-MRSA in the USA.17,18,22

MRSA isolates with antibiotic resistance profiles IV, VI and XIII carry SCC type IA and so are presumed to belong to the Iberian clone. All resistance profile XIII isolates have teicoplanin MICs equal to 4 mg/L or more and must be classified as glycopeptide-intermediate S. aureus (GISA) according to the CA-SFM recommendations. It has been reported that GISA outbreaks occurring since 1996 in many French hospitals were due to strains related to this clone.23

Most of HA-MRSA from the USA and Japan have SCC type II or III.7,8 In Europe, two previous studies have reported such strains: EMRSA-16, the most epidemic MRSA in the UK, carries SCC type II, and isolates related to the Brazilian–Hungarian clone from Portugal and Hungary carry SCC type III,6,8 but in this study, no MRSA antibiotic-resistance profile was found to be associated with these SCC types.

There was a good concordance between antibiotic-resistance profile and PFGE profile (i.e. isolates with the same resistance profile belong to the same PFGE cluster), other than for isolates expressing resistance profiles X, XI and XII. These results suggest that, in our hands, results of antibiotyping fit well with molecular typing methods used to delineate epidemic strains.

Comparisons of results from the two different PFGE systems used for MRSA surveillance in our hospital must be interpreted cautiously. However, since all MRSA isolates with resistance profile VII, which has become very prevalent, were located by one method in cluster N1 and by another in cluster C1, we assume that all isolates in these two PFGE clusters are genetically related. All MRSA with SCC types I or IA belong to this genetic background. However, we have shown that this same genetic background contains SCC type IVA strains, but, unlike SCC types I or IA, SCC types IV and IVA are not restricted to this genetic background. Among MRSA isolated before 1998, gentamicin-resistant isolates with SCC type IVA were all grouped in the well-separated cluster N2; SCC type IVA strains isolated since 1998 are scattered about clusters C1 and C2 and also represent single isolates not related to these clusters.

In France, genetic relatedness between recently isolated gentamicin-susceptible and some multi-resistant, gentamicin-resistant MRSA have been reported previously.13 A common genetic background has also been described in an evolutionary model for MRSA clonal complex 8 (CC8), which includes the Archaic, Iberian and Brazilian clones and several European MRSA clones with SCC type IV or IVA.10 Interestingly, MRSA isolates with resistance profile VII and SCC type IVA have a multilocus sequence type 8 (H. de Lencastre and D. Oliveira, personal communication). The diversity of genetic backgrounds of MRSA with SCC types IV or IVA has been underlined previously for HA-MRSA as well as CA-MRSA isolates.6,10,24

Our results from a single hospital fully agree with these reports.

In conclusion, since 1997 the epidemiological background of MRSA in our hospital has been characterized by an increased prevalence of SCC type IV or IVA HA-MRSA isolates. Although the greater ability of isolates expressing antibiotic resistance profile VII to cause epidemics cannot be conclusively linked to their acquisition of SCC type IVA, nonetheless, this SCC type is present in the majority of recent epidemic MRSA strains. Since 1997, types IV and IVA MRSA, expressing less resistance to other antibiotics than to β-lactams, have emerged independently from several different genetic lineages of S. aureus.

References

SCC types and antibiotic resistance profiles in MRSA isolates


