Genetic Analysis of Glycopeptide-Resistant Staphylococcus epidermidis Strains from Bone and Joint Infections

Julie Cremniter, Valérie Sivadon-Tardy, Charlotte Cauliez, Thomas Bauer, Raphaël Porcher, Alain Lortat-Jacob, Philippe Pirou, Thierry Judet, Philippe Aegerter, Jean-Louis Herrmann, Jean-Louis Gaillard, Martin Rottman

Glycopeptide-resistant Staphylococcus epidermidis (GRSE) strains are of increasing concern in bone and joint infections (BJIs). Using multilocus sequence typing and multilocus variable-number tandem repeat analysis, we show that BJI-associated GRSE strains are genetically diverse but arise from related, multiresistant hospital sequence types (STs), mostly ST2, ST5, and ST23.

Staphylococcus epidermidis is a major nosocomial pathogen causing a variety of device-related infections in humans. S. epidermidis can develop antibiotic resistance and also form biofilm on implanted medical devices. In most countries, 75 to 90% of all hospital S. epidermidis isolates are resistant to methicillin. Resistance to fluoroquinolones, erythromycin, clindamycin, gentamicin, rifampin, tetracycline, and chloramphenicol is also frequent. Multilocus sequence typing (MLST) analysis suggests that multidrug resistance in S. epidermidis is associated with a small number of related clones, mostly belonging to ST2 and related sequence types (STs). Recent studies report the recovery of increasing numbers of glycopeptide-resistant S. epidermidis (GRSE) strains around the world. Glycopeptide resistance has been detected in a large number of S. epidermidis isolates from patients with bone and joint infections (BJIs) and is a matter of particular concern in orthopedic surgery. Most of the GRSE isolates recovered are resistant to methicillin, and many are also resistant to other antibiotics widely used to treat staphylococcal BJIs, including rifampin or clindamycin. However, nothing is known about the genetic background of the GRSE strains involved in BJIs and the epidemicity of these strains.

We used both MLST (17–19) and multilocus variable-number tandem repeat analysis (MLVA) (20–22) to study S. epidermidis strains from BJIs. We found that the shift of these strains toward greater resistance to glycopeptides is a widespread phenomenon occurring in many STs rather than the result of the spread of a small number of GRSE strains. However, most BJI GRSE strains emerge from nosocomial, multiresistant STs (e.g., ST2, ST5, ST23), making them a serious problem in orthopedics.

The study was conducted in the Orthopedic Department of the Groupe Hospitalier Raymond Poincaré—Ambroise Paré (France), a reference center for the management of BJIs in the greater Paris area. We included all infecting S. epidermidis strains recovered from BJI cases between January 2003 and December 2005. An infecting strain was defined as a single strain (i.e., isolates having the same colony morphology, antibiotic susceptibility patterns, and sodA sequences) isolated from ≥2 independent intraoperative samples following a single surgical procedure. Patients could be included several times as cases if these criteria were fulfilled and if at least 2 months elapsed between surgical procedures.

All isolates were identified to the species level by partial sodA sequencing (23). A disk diffusion method was used for antibiotic susceptibility testing according to the EUCAST 2012 recommendations (http://www.eucast.org/antimicrobial_susceptibility_testing/); strains with inconclusive results for resistance to methicillin were further studied by testing for the mecA gene (24). The MICs of vancomycin and teicoplanin were determined with the 2-fold agar dilution method (3), and values were interpreted according to EUCAST criteria (25). A GRSE strain was defined as any strain resistant to teicoplanin (MIC value of >4 mg/liter) and/or vancomycin (MIC value of >4 mg/liter). A glycopeptide-sensitive S. epidermidis (GSSE) strain was defined as any strain susceptible to both teicoplanin (MIC value of ≤4 mg/liter) and vancomycin (MIC value of ≤4 mg/liter). A GRSE case was defined as any case from which at least one GRSE strain was isolated. A GSSE case was defined as any case from which only GSSE was isolated.

MLST was performed using the scheme developed by Thomas et al. (17). Sequences were compared with the sequences of known alleles for each locus in the MLST database (http://www.mlst.net), and the resulting seven-digit profiles, defining STs, were used to interrogate the database for matches. Clonal relationships were analyzed with the BURST clustering algorithm (26, 27), using eBURST version 3 (http://eburst.mlst.net), and included all available STs from the MLST database. MLVA was performed using the published primers Se1, Se2, Se3, and Se4 (22), and the following three additional primers were chosen for enhanced discriminatory power (this study), with sequences selected from the Microorganism Tandem Repeats Database (http://www.minisatellites.u-psud.fr) (28): Se6APR (forward/reverse, AGACATTTTAGCATT TTACCGATTG/CATTGGGAGCATCACCCTTT), Se7APR (TG...
GTTCAGTGGGCATAAG/CAGCAATGAGTCTGGGACAA), and Se8APR (TGAAGCACCAGATGTCTTGAAATGATTGTT). START2 software was used for lineage assignment (26). Minimum spanning trees were constructed with the MLST Data Analysis Web tool provided online at the PubMLST website (http://www.pubmlst.org/analysis).

Fisher’s exact test was used to evaluate the significance of 2-by-2 contingency tables. Wilcoxon’s signed-rank test was used for quantitative data. Correlations between vancomycin and teicoplanin MICs were assessed with Spearman’s signed-rank test. Values of 0.05 were considered to be significant.

We included 75 BJI cases (70 patients) with the isolation of at least one infecting S. epidermidis strain (Table 1); 52 cases had one S. epidermidis strain, 21 had two, and two had three. Thus, 100 different strains were isolated and included in the analysis. Thirty-eight (50.7%) cases involved at least one GRSE strain (GRSE cases) and 37 (49.3%) only GSSE (GSSE cases). GRSE cases tended to be older than GSSE cases (median age, 60.5 compared to 49 years), but the difference was not statistically significant (P = 0.122). The other characteristics studied, as follows, did not differ between the two groups (Table 1): the sex ratio, the nature of the BJI, a coinfection with other bacterial species, the time elapsed since the first procedure, and the number of previous interventions at the same location.

Teicoplanin and vancomycin MIC values were determined for all infecting strains (n = 100) recovered from the 75 BJI cases (Fig. 1), and 47% (47/100) of the strains were GRSE. All GRSE strains were resistant to teicoplanin, with most (37/47, 78.7%) showing a teicoplanin MIC value of 8 mg/liter (Fig. 1), but all GRSE strains except one (vancomycin MIC value of 8 mg/liter) were susceptible to vancomycin. However, for all strains included, the MIC values for vancomycin and teicoplanin were positively correlated (Spearman’s signed-rank test, P < 10⁻⁴).

GRSE strains were significantly more likely than GSSE strains to be resistant to multiple antimicrobial agents, with a median (interquartile range [IQR]) of 9 (4.5 to 10.5) compared to 4 (2 to 9) associated resistance markers, respectively (P = 0.0012). Resistance markers significantly associated with glycopeptide resistance were methicillin (GRSE compared to GSSE, 93.6% compared to 47.2%; P = 0.004), erithromycin (61.7% compared to 35.8%; P = 0.016), kanamycin (68.1% compared to 45.3%; P = 0.027), tobramycin (63.8% compared to 41.5%; P = 0.029), gentamicin (55.3% compared to 32.1%; P = 0.026), and tetracycline (29.8% compared to 11.3%; P = 0.026). Resistance to penicillin, rifampin (44.6% compared to 35.8%; P = 0.4), lincomycin, pristinamycin, fosfomycin, fucidic acid, and trimethoprim-sulfamethoxazole was not significantly associated with glycopeptide resistance.

Ninety-seven strains were successfully typed by MLST and clustered into 33 STs (Fig. 2): GRSE strains (n = 46) belonged to 16 STs, mostly ST5 (n = 14), ST23 (n = 10), and ST2 (n = 6); GSSE strains (n = 51) belonged to 25 STs, mostly ST2 (n = 12),

### Table 1: Characteristics of cases

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR) age, in yrs</td>
<td>All cases (n = 75) GSSE cases (n = 37) GRSE cases (n = 38)</td>
</tr>
<tr>
<td>Sex ratio, male to female (95% confidence interval)</td>
<td>2.6 (1.6–4.6) 2.7 (1.4–6.9) 2.5 (1.3–5.9)</td>
</tr>
<tr>
<td>Prosthesis-associated BJI</td>
<td>42 (56.0) 22 (59.5) 20 (52.6)</td>
</tr>
<tr>
<td>No. of cases (%) with coinfection with other species</td>
<td>20 (26.7) 9 (24.3) 11 (28.9)</td>
</tr>
<tr>
<td>Median (IQR) time since the first procedure, in mo</td>
<td>24 (6–72) 24 (2.5–52) 17 (6.25–150)</td>
</tr>
<tr>
<td>Median (IQR) no. of previous procedures b,c</td>
<td>2 (1–3) 2 (1–3) 2 (1–3.75)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 (59.5)</td>
</tr>
<tr>
<td>11 (28.9)</td>
</tr>
<tr>
<td>17 (6.25–150)</td>
</tr>
<tr>
<td>2 (1–3.75) 0.734</td>
</tr>
<tr>
<td>GRSE compared to GSSE cases.</td>
</tr>
<tr>
<td>GRSE compared to GSSE cases.</td>
</tr>
<tr>
<td>GRSE compared to GSSE cases.</td>
</tr>
<tr>
<td>GRSE compared to GSSE cases.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR) no. of previous procedures b,c</td>
<td>2 (1–3) 2 (1–3) 2 (1–3.75)</td>
</tr>
</tbody>
</table>

**Figure 1** Distribution of teicoplanin and vancomycin MIC values in the S. epidermidis strains studied. Threshold values for resistance to teicoplanin (continuous line) and to vancomycin (broken line) are indicated. All GRSE strains were resistant to teicoplanin and all but one (vancomycin MIC value = 8 mg/liter) were susceptible to vancomycin. The MICs of vancomycin and teicoplanin were positively correlated (Spearman’s signed-rank test, P < 10⁻⁴).
Overall, 44 of the 46 GRSE strains (compared to 42 of the 51 GSSE strains; \( P = 0.054 \)) belonged to the main clonal complex, with a particularly high proportion of GRSE in ST23 (58.8%) and ST5 (70%). The number of resistance markers was significantly higher for GRSE than GSSE subsets within the main clonal complex (median [IQR], 9 [5.75 to 11] and 5.5 [2.25 to 9.75] resistance markers, respectively; \( P = 0.015 \)).

The strains of ST2, ST5, and ST23 (GRSE, \( n = 30 \); GSSE, \( n = 30 \)) were further analyzed using MLVA. The minimum spanning tree analysis (FIG 3) revealed distinct genetic clusters for each ST type, with the GRSE strains showing a higher degree of genetic diversity compared to the GSSE strains.

**FIG 2** eBURST analysis of the strains typed by MLST. ST-numbered circles represent the 33 STs identified among the 97 typeable strains. The size of the circle indicates the number of GRSE (black) and GSSE (white) strains. STs that are directly connected differ at only one single locus. STs previously reported elsewhere and not found in the present study are shown as light-gray dots (population snapshot, http://sepidermidis.mlst.net/). The asterisk indicates the GRSE strain resistant to vancomycin (MIC value = 8 mg/liter).

**FIG 3** Minimum spanning tree analysis of ST2, ST5, and ST23 strains typed by MLVA. Each circle denotes a particular MLVA profile, and the size of the circle indicates the number of GRSE (black) and GSSE (white) strains displaying that particular profile. The lines denote connections between MLVA profiles. The asterisk indicates the GRSE strain resistant to vancomycin (MIC value = 8 mg/liter).
were subtyped by MLVA (Fig. 3). This led to the identification of 28 profiles forming three major clusters, consistent with the MLST data (ST2, 11; ST5, 10; ST23, 7). Twelve profiles were associated with GRSE strains only, six with both GRSE and GSSE strains, and 10 with GSSE strains only (Fig. 2). GRSE strains thus displayed 18 distinct profiles and GSSE strains 16. Only three MLVA profiles were shared by more than two GRSE strains: profile 3 (ST23, 6 strains), profile 21 (ST5, 4 strains), and profile 22 (ST5, 4 strains). GRSE strains of profiles 3 and 21 displayed various antibiotic resistance patterns, whereas the four GRSE strains of profile 22 were undistinguishable from each other (same MIC values for glycopeptides and same antibiotic resistance pattern or differing by only one marker). Thus, four of the 38 GRSE cases (4 patients) were infected with the same ST5 strain, with profile 22 (Fig. 4). These findings show that the GRSE strains isolated from BJIs in the Paris area are representative of the populations of multidrug-resistant strains circulating in hospitals worldwide (10, 11, 29–33). Almost 95% of these strains belong to the STs of the main clonal complex, principally ST2, ST5, and ST23, and most of these strains are resistant to numerous antibiotics, such as oxacillin, macro-

FIG 4 MLVA subtyping of ST2, ST5, and ST23 strains. Dendrogram built with START2 software, using the unweighted-pair group method using average linkages (UPGMA) method. For each strain belonging to STs 2, 5, and 23, ST, MLVA digit, MLVA number, patient identifier (anonymized by single-letter coding) for patients with multiple ST2, ST5, and ST23 strains isolated and analyzed, vancomycin MIC, and teicoplanin MIC are shown. Resistant strains according to EUCAST 2012 are shown in white against a black background. *, only patients with more than two S. epidermidis isolates are shown; V, vancomycin; T, teicoplanin.
lides, quinolones, and fucidic acid. By combining MLST and MLVA, we show that this population of strains displayed substantial genetic diversity. Indeed, they belonged to 17 different STs, and the subtyping of ST2, ST5, and ST23 strains by MLVA identified 20 different profiles. Phenotypic profiles of resistance to antibiotics, including glycopeptides, were often heterogeneous, further evidence of the diversity of the strains. In most cases, resistance to glycopeptides appeared to be an individual phenomenon repeatedly emerging in the main clonal complex, rather than the result of the spread of a small number of clones. These findings are consistent with those of previous coagulase-negative Staphylococcus typing studies, which reported a decrease in susceptibility to teicoplanin in isolates from various clinical settings with a broad strain diversity (34, 35).

Our findings and published results, together with the positive correlation between MIC values for vancomycin and teicoplanin, suggest that the most likely scenario is that of GRSE strain emergence as a consequence of the increasingly widespread use of glycopeptides (35, 36). Sieradzki et al. have shown that strains of S. epidermidis isolated before the introduction of antibiotics can express heteroresistance to teicoplanin (15, 37) and that, in laboratory conditions, vancomycin can select bacteria with higher MICs for teicoplanin (37). Schwalbe et al. showed that there is a relationship between the cumulative dose of glycopeptides administered to a patient and the emergence of resistance to these antibiotics (38). A number of phenotypic traits have been reported in GRSE strains, including cell wall thickening and a tendency of bacterial cells to form cellular aggregates (16, 37, 39), but no specific genetic determinant of resistance to glycopeptides has yet been identified. GRSE strains thus appear to be variants that emerge under glycopeptide selection pressure, from populations circulating in the hospital environment.

However, a small number of patients in our series were infected with GRSE strains from ST5 that could not be distinguished by MLVA and had identical antibiotic resistance profiles. This may reflect the limitations in terms of discrimination power of MLVA for hyperclonal populations. Alternatively, GRSE may circulate among patients, as recently reported for strains of S. epidermidis resistant to linezolid (40). Although this phenomenon appears to make only a minor contribution to the overall emergence of glycopeptide resistance in S. epidermidis, it nevertheless warrants keeping GRSE populations under surveillance in orthopedic wards and in hospitals more generally. Either through transmission of resistant clones or through acquisition of resistance in previously susceptible strains, the emergence of glycopeptide-resistant strains in S. epidermidis is yet another motive to promote strict antibiotic stewardship practices to preserve the activity of glycopeptides.

REFERENCES


