Decreased susceptibility to teicoplanin and vancomycin in coagulase-negative staphylococci isolated from orthopedic device-associated infections

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**Keywords:** Glycopeptides, vancomycin, teicoplanin, coagulase-negative staphylococci, Staphylococcus epidermidis, bone and joint infections.

**Running head:** Glycopeptide resistance and CoNS in orthopedics
We studied 315 coagulase-negative Staphylococcus strains recovered prospectively during 240 surgical procedures (206 subjects) from proven or suspected device-associated bone and joint infections. Sixteen strains (5.1%) had decreased susceptibility to glycopeptides: 15 (12 S. epidermidis, 2 S. capitis, 1 S. haemolyticus) to teicoplanin alone (minimal inhibitory concentration [MIC] = 16 mg/l, n=9; MIC = 32 mg/l, n=6) and one (S. epidermidis) to both teicoplanin and vancomycin (MIC, 16 and 8 mg/l, respectively). Decreased susceptibility to teicoplanin was more prevalent in “infecting” strains (i.e., strains recovered from ≥ 2 distinct intraoperative samples) (8.1% [12/149] vs 2.4% [4/166] in “contaminants”, p=0.022). All S. epidermidis strains with decreased susceptibility to teicoplanin were resistant to methicillin (13/13 vs 112/173 [64.7%] for S. epidermidis strains susceptible to teicoplanin, p=0.021).
Coagulase-negative staphylococci (CoNS), and especially *Staphylococcus epidermidis*, are major nosocomial pathogens causing a variety of device-related infections in humans (25). In most hospitals, 60 to 70% of CoNS isolates are resistant to methicillin (20). This leads to the frequent use of glycopeptide antibiotics for treatment of CoNS infections (6, 19, 21). The first reports of CoNS with decreased susceptibility to glycopeptides were published in the United States in the late 1970s (13), and shortly afterwards in European countries (2, 11, 21). These strains usually show resistance to methicillin and most other anti-staphylococcal agents (19), with the exception of the new agents tigecycline, linezolid and daptomycin (5). Unlike *Staphylococcus aureus* (14, 18), CoNS resistance to glycopeptides is almost exclusively to teicoplanin (15, 21, 24). The mechanisms involved are unclear, but CoNS with decreased susceptibility to glycopeptides show cell wall thickening and tend to form cellular aggregates (7, 10, 15).

Several studies have reported an increase in the prevalence of CoNS with decreased susceptibility to glycopeptides (8, 24). However, these studies mainly included strains from patients with CoNS bacteremia and little information was provided about the initial source of infection. To our knowledge, no prospective study has been carried-out in orthopedic surgery. Resistance of CoNS to glycopeptides may, however, be a major problem in this context. CoNS are the main cause of prosthetic joint infections and other device-related bone and joint infections (BJIs) (12, 16). Most strains are resistant to methicillin and other drugs commonly used for the treatment of BJIs, leading to an increase in the use of glycopeptides (23). It is thus important to know the current prevalence of CoNS strains with decreased glycopeptide susceptibility in orthopedic surgery. This was the aim of this 3-year surveillance study.

We carried out a prospective study of all CoNS isolates recovered intraoperatively from cases or suspected cases of BJI between January 2003 and December 2005 in the Orthopedic Department of the Raymond Poincaré hospital (Garches, France), a national...
reference center for the management of BJIs in France. Tissue samples obtained intraoperatively were analyzed microbiologically as previously described (16). We only included CoNS isolates from patients with at least three independent samples collected during the same surgical procedure. Patients could be included several times if the above criteria were fulfilled. Mixed infections (e.g., isolation of infecting organisms other than CoNS) were not excluded. The “CoNS group” was identified using Gram staining and the following tests: catalase, Slidex latex agglutination (BioMérieux) and tube coagulase (Bio-Rad, Marnes la Coquette, France). CoNS isolates were then identified to species level by partial sodA sequencing, as described elsewhere (17). The susceptibility of strains to 15 antibiotics was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) (4) using the following disks (content in µg): penicillin (6 µg), oxacillin (5 µg), ofloxacin (5 µg), erythromycin (15 µg), lincomycin (15 µg), pristinamycin (15 µg), rifampin (30 µg), gentamicin (10 UI), kanamycin (30 µg), tobramycin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), fosfomycin (50 µg) vancomycin (30 µg) and teicoplanin (30 µg). Results were interpreted according to the breakpoints of the Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM) (4). Susceptibility to oxacillin was used for determining methicillin resistance; strains with inconclusive responses were further tested by mecA detection, performed as described (9). CoNS isolates recovered from various samples but belonging to the same species (identical partial sodA sequence) were deemed to be the same strain if they had the same colony morphology and an identical antibiotic susceptibility pattern (1). A strain was defined as an “infecting strain” if it was recovered from ≥2 distinct peroperative samples (22); a strain not fulfilling this criterion was defined as a “contaminant”. All CoNS strains were cryo-preserved after initial culture.

Strains with decreased susceptibility to glycopeptides were detected by screening with the E-test (teicoplanin and vancomycin E-test strips, AB Biodisk Dalvägen, Solna, Sweden).
Mueller-Hinton agar plates were inoculated by swabbing (Difco Laboratories, Cockeysville, MD, USA) with a 0.5 McFarland standard bacterial suspension, and incubated at 37°C for 24h. All strains, for which the minimal inhibitory concentration (MIC) of vancomycin and/or teicoplanin was ≥ 4 mg/l with the E-test, were further assessed using the two-fold agar dilution method with teicoplanin and vancomycin concentrations ranging from 0.25 to 64 mg/l. Mueller-Hinton agar plates were inoculated with 2 µl (10^4 CFU) of each strain using a Steers replicator and incubated for 24h at 37°C. The reference strains S. aureus ATCC 25923 and S. epidermidis CIP 105777 (=ATCC 35984), were controls. MIC values were defined as the lowest concentration of antibiotic that completely inhibited bacterial growth. Vancomycin and teicoplanin MIC values were interpreted according to the 2006 recommendations of the Clinical and Laboratory Standards Institute (CLSI) (3), which classify CoNS strains as “susceptible”, “intermediate” or “resistant”, as follows: i) teicoplanin: MIC values of ≤ 8 mg/l, 16 mg/l and ≥ 32 mg/l, respectively; ii) vancomycin: MIC values of ≤ 4 mg/l, 8-16 mg/l and ≥ 32 mg/l, respectively. A strain with decreased susceptibility to glycopeptides was any strain considered to be “intermediate” or “resistant” to teicoplanin and/or vancomycin according to the above definitions. The chi-squared test (with Yates’ correction for expected frequencies <5) was used for comparisons, and p-values of <0.05 were considered to be statistically significant.

A total of 240 surgical procedures (206 patients) yielding CoNS were included prospectively. The patients (153 males and 53 females) were between 19 and 96 years (mean age, 56.6 years). Of these patients, 177 underwent a single procedure, 26 underwent two procedures, 1 underwent three procedures, and 2 underwent four procedures. Clinical data were available for 170 surgical procedures: 103 (60.6%) were revision arthroplasty surgery (hip, 80; knee, 23), and 67 (39.4%) were for other BJI. A total of 315 CoNS strains were recovered intraoperatively: 186 S. epidermidis (59.0%), 48 S. capitis (15.2%), 27 S. warneri
(8.6%), 15 S. lugdunensis (4.8%), 11 S. hominis (3.5%), 7 S. haemolyticus (2.2%), 7 S. caprae (2.2%), 4 S. pasteuri (1.3%), 4 S. simulans (1.3%), 3 S. pettenkoferi (1.0%), 1 S. schleiferi (0.3%), 1 S. cohnii (0.3%), 1 S. pseudintermedius (0.3%). Using the E-test, we found that 147 strains had a MIC ≥ 4 mg/l for vancomycin and/or teicoplanin. We used the agar dilution method to further assess these 147 strains: 131 were susceptible to both teicoplanin (MIC=1 mg/l, n=2; MIC=2 mg/l, n=11; MIC=4 mg/l, n=54; MIC=8 mg/l, n=64) and vancomycin (MIC=1 mg/l, n=10; MIC=2 mg/l, n=88; MIC=4 mg/l, n=33), 15 had decreased susceptibility to teicoplanin only (MIC=16 mg/l, n=9; MIC=32 mg/l, n=6) and one (S. epidermidis) had decreased susceptibility to both teicoplanin (MIC=16mg/l) and vancomycin (MIC=8mg/l). The MIC of teicoplanin was ≥ 8 mg/l using the E-test for the 16 strains with decreased susceptibility to teicoplanin with the agar dilution method (data not shown).

Thus, 16 (5.1%) of the 315 CoNS strains studied had decreased susceptibility to teicoplanin, i.e. 10 (3.2%) intermediate strains (9 S. epidermidis, 1 S. haemolyticus) and 6 (1.9%) resistant strains (4 S. epidermidis, 2 S. capitis) (Table 1). Decreased susceptibility to teicoplanin was more prevalent in infecting strains than in contaminants (12/149 [8.1%] vs 4/166 [2.4%], p=0.022). Among the 13 S. epidermidis strains with decreased susceptibility to teicoplanin, 13 (100%) were resistant to oxacillin (vs 112/173 [64.7%] for S. epidermidis strains susceptible to teicoplanin, p=0.021) (Table 2). Other resistance markers significantly associated with decreased susceptibility to teicoplanin in the subset of S. epidermidis strains included resistance to lincomycin, rifampin, and fosfomycin (Table 2). We did not test the new antimicrobial agents tigecycline, linezolid and daptomycin. However, studies of the susceptibility to these newer agents of CoNS with decreased susceptibility to glycopeptides have reported MIC₉₀ (minimum inhibitory concentration at which 90% of isolates are inhibited) close to the CLSI breakpoints (5). Given the pharmacodynamic profile of the
periprosthetic tissue, the determination of MIC could be advised prior to the initiation of a
treatment using these molecules.

The orthopedic surgery department of Garches hospital is a French referral center for
the management of patients with BJIs. Patients are mainly from the greater Paris area, but also
come from other regions of France. Our data are thus a good indication of current situation
regarding sepsis in orthopedics in France. We found a relatively low prevalence of resistance
to glycopeptides in the CoNS strains studied, with 5.1% of strains showing decreased
susceptibility to teicoplanin and 1.9% being resistant; only one strain had decreased
susceptibility to both teicoplanin and vancomycin (MIC, 16 and 8 mg/l, respectively). These
data are similar to those of an Italian study of bacteremia caused by CoNS, which reported
that the percentage of strains resistant to glycopeptides was 2% in surgical wards (21).
However, our figures are considerably lower than those reported in recent European studies,
which demonstrated an alarming increase in resistance to teicoplanin in CoNS strains since
the beginning of this century. In a study of 1,337 CoNS isolates recovered from patients with
bacteremia from 2001 to 2004 in Greece, the prevalence of teicoplanin-resistant strains
increased from 0% in 2001-2002 to 6.4% in 2003-2004 (8). In a recent retrospective French
study of 1,039 CoNS isolates recovered in routine practice from 2000 to 2004, the prevalence
of decreased susceptibility to teicoplanin was 7.2% in 2000, 17.2% in 2001, and exceeded
30% in 2002, 2003 and 2004 (24). These discrepancies may be explained by differences in the
nature of specimens, the clinical context of included patients and the infectiousness of the
isolated stains. For example, CoNS resistance to glycopeptides has been shown to be 4-times
higher in intensive care units than in surgical wards (21).

The results of our study should not lead to a false sense of security and less vigilant
surveillance of the prevalence of resistance to glycopeptides, in particular to teicoplanin, in
CoNS in orthopedic surgery. On the one hand, our data show that the resistance to teicoplanin
is 3.4 times more frequent in infecting strains than in contaminants. The prevalence of
teicoplanin resistance thus seems much higher in truly pathogenic strains similar to that
reported by Tacconelli et al. in intensive care units (21). On the other hand, the CoNS
population is clearly shifting towards greater resistance to glycopeptides, probably as a result
of pressure due to the increase in use of these molecules in recent years (19). This shift is
particularly marked in *S. epidermidis*, the main cause of device-related BJIs worldwide (12).

We found that 100% of *S. epidermidis* strains with decreased susceptibility to teicoplanin
were resistant to methicillin and that this resistance was frequently associated with other
markers, such as resistance to as lincomycin, rifampin or fosfomycin. These results are of
particular concern as rifampin and lincomycin/clindamycin are widely used for treatment of
CoNS BJIs. Moreover, they suggest the selection of subclones less susceptible to
glycopeptides from the multi-resistant *S. epidermidis* clones currently circulating in hospitals.

Genotyping studies using pulsed field gel electrophoresis (15) or ribotyping (21) have shown
a broad diversity of *S. epidermidis* strains with decreased susceptibility to glycopeptides.
These findings need to be confirmed in the particular context of device-associated BJIs, using
techniques, such as multi-locus sequence typing, that allow better analysis of the relationship
between the bacterial populations involved.

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Table 1. Prevalence of strains with decreased susceptibility to teicoplanin.

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. (%) of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>All strains (n=315)</td>
<td>10 (3.2)</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (n=186)</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td>Other CoNS (n=129)</td>
<td>1&lt;sup&gt;c&lt;/sup&gt; (0.8)</td>
</tr>
<tr>
<td>Infecting strains&lt;sup&gt;a&lt;/sup&gt; (n=149)</td>
<td>7 (4.7)</td>
</tr>
<tr>
<td>Contaminants (n=166)</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Resistant to methicillin (n=149)</td>
<td>10 (6.7)</td>
</tr>
<tr>
<td>Susceptible to methicillin (n=166)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> ≥ 2 distinct positive samples.

<sup>b</sup> I, MIC = 16mg/l; R, MIC ≥ 32mg/l; I or R (decreased susceptibility to teicoplanin), MIC ≥ 16mg/l.

<sup>c</sup> *S. haemolyticus*.

<sup>d</sup> Two *S. capitis* strains (two subjects).

<sup>e</sup> Infecting strains vs contaminants, p=0.022.

<sup>f</sup> Abbreviations. I, intermediate; R, resistant.
Table 2. Resistance markers associated with decreased susceptibility to teicoplanin in *S. epidermidis* strains.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. (%) of resistant strains&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I/R to teicoplanin (n=13)</td>
<td>S to teicoplanin (n=173)</td>
</tr>
<tr>
<td>Methicillin</td>
<td>13 (100)</td>
<td>112 (64.7)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>10 (76.9)</td>
<td>88 (50.9)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7 (53.8)</td>
<td>66 (38.1)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>9 (69.2)</td>
<td>75 (43.3)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>7 (53.8)</td>
<td>35 (20.2)</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>2 (15.4)</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>9 (69.2)</td>
<td>54 (31.2)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4 (30.8)</td>
<td>27 (15.6)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>7 (53.8)</td>
<td>42 (24.3)</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>7 (53.8)</td>
<td>91 (52.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Intermediate and resistant strains, according to the 2009 recommendations of the CA-SFM committee<sup>(1, 4)</sup>. Abbreviations. S, susceptible; I, intermediate; R, resistant.