Changing susceptibilities of coagulase-negative staphylococci to teicoplanin in a teaching hospital

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The susceptibility of two collections of coagulase-negative staphylococci (CNS) isolated from clinical specimens for teicoplanin and vancomycin were compared. They comprised 91 and 101 isolates, collected in 1985 and 1994 respectively, from different departments of a teaching hospital. MICs of vancomycin and teicoplanin were determined by a modified Etest method. Additionally, a disc diffusion test was performed for teicoplanin. All isolates were susceptible to vancomycin (MIC ≤ 4 mg/L). Two of the 91 isolates collected in 1985 were intermediate to teicoplanin (MIC between 8 and 32 mg/L), whereas in 1994 the number of intermediate isolates was 20 out of 101 (P < 0.01). The correlation between MICs, as determined by the modified Etest assay, and disc diffusion zones was poor (r = −0.35). Results show that resistance to teicoplanin in CNS has increased in the study hospital over a period of 9 years. This increase is likely to be correlated with the introduction of teicoplanin. Furthermore, a disc diffusion method does not appear to be the first method of choice for detection of strains of CNS with diminished susceptibility to teicoplanin.

Introduction

Coagulase-negative staphylococci (CNS) are a leading cause of bloodstream infections in tertiary care hospitals. Infections with CNS occur in specific groups of patients, including neonates, patients with neutropenia and patients with indwelling prosthetic devices. Owing to resistance to β-lactam antibiotics, the antibiotic of choice for treatment of infections with CNS is usually a glycopeptide, vancomycin or teicoplanin. Resistance of CNS to these antibiotics has been reported, but is usually limited to decreased efficacy of teicoplanin against Staphylococcus haemolyticus and Staphylococcus epidermidis, although resistance to glycopeptides has been found in other species of CNS as well. Bloodstream infections with teicoplanin-resistant CNS occur, and may have serious clinical consequences. In an in-vitro pharmacokinetic model in which CNS were exposed to teicoplanin, teicoplanin-resistant strains were selected. Selection of teicoplanin resistance in vivo during treatment with both vancomycin and teicoplanin has been described recently in a patient with non-Hodgkin’s lymphoma.10 Thus, active surveillance of resistance of CNS to these agents is recommended.

The Leiden University Medical Center is a 867 bed tertiary care hospital. Patients are predominantly from the vicinity of the hospital, but approximately 30% are from hospitals in other parts of the country or from abroad. According to the hospital pharmacy’s records, a total amount of 1688 g of vancomycin was prescribed in 1985, whereas teicoplanin was not used at that time. In 1994, 1272 g of vancomycin and 972 g of teicoplanin were administered in the hospital.

In the present study susceptibilities of CNS from the Leiden University Medical Center to both glycopeptides were compared for isolates from 1985 and 1994. MICs were determined using a modified Etest method, which was validated by comparison with the standard Etest method.

Materials and methods

Isolates

A total of 201 CNS isolates from clinical specimens were included in this study. Ninety-seven isolates were from a collection of CNS isolates from 1985, previously studied by Mouton et al.11 In that study, 372 CNS isolates were collected from four departments of the Leiden University Medical Center.
Medical Center (designated in that study as ‘Hospital 1’), including cardiology, surgery intensive care, neurosurgery and thoracic surgery. In the present study 97 isolates from 1985 were used, including the first 25 consecutive isolates from each of these four departments, except for the thoracic surgery department, from which only 22 isolates were available. The remaining 104 isolates were collected in 1994 in the same hospital, and were from the following departments: internal medicine (including cardiology, \( n = 33 \)), surgical departments (excluding intensive care, \( n = 15 \)), surgery intensive care (\( n = 19 \)), neurosurgery (\( n = 10 \)), thoracic surgery (\( n = 9 \)), paediatrics (inclusive neonatology, \( n = 16 \)); one isolate was from neurology and another from a post-mortem. All isolates were from patients suspected of having septicaemia or deep infections on the basis of the Centers for Disease Control criteria of nosocomial infections.\(^{12}\) Only one isolate per patient was included.

Isolates were identified as \textit{S. epidermidis} as previously described.\(^{13}\) Isolates not belonging to \textit{S. epidermidis} were further identified to species level using API Staph (bioMérieux, Marcy l’Etoile, France).

**Susceptibility testing**

Susceptibilities to vancomycin and teicoplanin were assessed by determination of the MIC of both drugs using an Etest (\textit{A B Biodisk}, Solna, Sweden) filter assay on Mueller–Hinton agar. This method has been described for MBC determination.\(^{14}\) A polycarbonate membrane filter (diameter 47 mm, pore size 0.2 \( \mu \text{m} \); Poretics, Livermore, CA, USA) was placed into the middle of a Mueller–Hinton agar plate (Becton Dickinson, Cockeysville, MD, USA). The surface of the filter was inoculated by soaking a swab in a bacterial suspension\(^{15}\) and streaking gently in four directions on the surface of the filter. An E test strip with either vancomycin or teicoplanin was applied to the filter in a predetermined position. MICs were read after incubation at 35°C for 24 h according to the manufacturer’s instructions.

A modified E test method was used in this study. The influence of the polycarbonate membrane filter on the test results was tested on a random selection of 20 CNS isolates by comparing MICs obtained using the Etest with these filters with those without these filters. Corresponding MICs were identical for all except four isolates, in which corresponding MICs differed by a factor of 2, which is within the accepted range of fluctuations of MIC measurements.

The collections from 1985 and 1994 were compared with respect to their susceptibilities to vancomycin and teicoplanin. All MICs were determined in duplicate on separate occasions, and the means of the duplicate MICs were used (Only isolates with a maximum difference of duplicate MICs of a factor of 2 for each of the two antibiotics were analysed further. Six isolates collected in 1985, and three from 1994, were excluded for this reason, resulting in 192 isolates which could be analysed). Critical MICs were used as defined by the National Committee for Clinical Laboratory Standards.\(^{16}\) The critical MICs of vancomycin were: >4 mg/L (susceptible), >4 mg/L, <32 mg/L (intermediate) and \( \geq 32 \) mg/L (resistant). Critical MICs of teicoplanin were: >8 mg/L, >8 mg/L, <32 mg/L and \( \geq 32 \) mg/L. \textit{Enterococcus faecalis} A TCC 29212 and \textit{Staphylococcus aureus} A TCC 29213, with MIC ranges of vancomycin of 1–4 mg/L and 0.5–2 mg/L, respectively, were used as controls.

Disc-diffusion sensitivities for teicoplanin were determined on Iso-Sensitest agar (CM 473, \textit{O xoid}, Basingstoke, U.K). Suspensions of fresh cultures of bacteria in sterile PBS were adjusted to a density of 0.5 McFarland and further diluted 1/30. The resulting suspension was applied on to agar plates in order to obtain semi-confluent growth, according to the Dutch national standards for susceptibility testing.\(^{17}\) Teicoplanin discs (30 \( \mu \text{g} \) ) (Becton Dickinson) were applied to the agar plates and inhibition zone diameters were measured after incubation at 35°C for 20 h. The procedure was performed in duplicate on separate occasions, and the means of the duplicates were used.

**Statistical analysis**

The \( \chi^2 \) test was used to compare the fraction of teicoplanin-intermediate isolates in both years. Spearman’s coefficient was calculated to determine the correlation between MICs and diameters of the inhibition zones.

**Results**

**Identification**

Eighty-three (85.6%) of the 97 CNS isolates from 1985 were identified as \textit{S. epidermidis}; the remaining isolates were assigned to \textit{S. haemolyticus} (2), \textit{Staphylococcus hominis} (2), \textit{Staphylococcus capitis} (3), \textit{Staphylococcus simulans} (2), \textit{Staphylococcus carnosus} (2) and \textit{Staphylococcus warneri} (1). For two isolates identification below genus level was not possible with the methods used. The distribution of the 104 isolates from 1994 over the species was: \textit{S. epidermidis} (88) (84.6%), \textit{S. haemolyticus} (9), \textit{S. hominis} (4), \textit{S. capitis} (1), \textit{Staphylococcus saprophyticus} (1) and \textit{Staphylococcus xylosus} (1).

**MICs**

The susceptibilities of 91 CNS isolates from 1985, and 101 isolates from 1994 are shown in the Table. All isolates from 1985 were susceptible to both vancomycin and teico-
Teicoplanin susceptibilities of CNS

<table>
<thead>
<tr>
<th></th>
<th>1985</th>
<th>1994</th>
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<tr>
<td><strong>Vancomycin</strong></td>
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<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>3.0</td>
<td>3.0</td>
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<tr>
<td>range</td>
<td>0.75–3.5</td>
<td>1.25–4.0</td>
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<td><strong>Teicoplanin</strong></td>
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<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>2.0</td>
<td>3.5</td>
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<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>5.0</td>
<td>11.8</td>
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<tr>
<td>range</td>
<td>0.056–10.0</td>
<td>0.5–16.0</td>
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### Discussion

This study shows the development of teicoplanin resistance over a 9 year period in a Dutch teaching hospital. The number of teicoplanin-intermediate CNS isolates increased from two out of 91 (2%) in 1985 to 20 out of 101 (20%) in 1994 (P < 0.01). In contrast, the susceptibility to vancomycin remained unchanged, with MICs in the susceptible range for all isolates tested (Table).

Many reports have commented on the diminishing sensitivity of CNS to teicoplanin, but not to vancomycin. In most reports the number of S. haemolyticus isolates resistant or intermediate susceptible to teicoplanin exceeded that of S. epidermidis allocated to these categories. In the present study three out of 11 S. haemolyticus and 17 out of 171 S. epidermidis were teicoplanin intermediate. Thus, the increase of resistance to teicoplanin was not exclusive for S. haemolyticus. The latter observation corresponds to that of Goldstein et al., who also found the majority of teicoplanin resistance in S. epidermidis, and less in S. haemolyticus. In the present study, we do not know whether the teicoplanin-intermediate isolates represented the same strain within each species, and it is not clear whether spread of such a strain occurred in the hospital. Nevertheless, the observation that all three S. haemolyticus isolates with decreased susceptibility to teicoplanin were from the same department fits with this hypothesis. In 1985 teicoplanin was not used in our hospital. It was introduced in 1993, and at present is the first choice in treatment of infections of CNS resistant to β-lactam antibiotics, with a total of 972 g used in 1994. Vancomycin is still used in the hospital, but the total amount used decreased from 1688 g in 1985 to 1272 g in 1994, which may explain why susceptibilities of
the Etest appeared to be an accurate method for showing that in the year after the introduction of teicoplanin, no statistically significant higher values than the latter method. In the same study, detection of CNS isolates resistant or intermediate to teicoplanin was not possible using disc diffusion. The latter observation has been confirmed by others. A possible explanation for the failure of detection of teicoplanin resistance in disc diffusion assays is the high molecular weight of teicoplanin, which results in poor diffusion in agar media. In the present study, a correlation coefficient of only -0.35 was calculated for the relation between log lowering of MICs of teicoplanin and the diameters of the inhibition zones.

In conclusion, the present report shows a decrease in susceptibility of CNS to teicoplanin in patients in a teaching hospital. Although removal of infected indwelling prosthetic devices is the major part of treatment, several patient groups will additionally be treated with glycopeptides. Because of the relatively high number of bacteraemias and other infections due to CNS in these patients in teaching hospitals, the observed decrease in susceptibility should be a cause of concern. The correlation between MICs of teicoplanin of CNS and results of disc diffusion is low, so the use of disc diffusion for detection of resistance to glycopeptides in CNS must be discouraged.

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References
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