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# Effects of Subinhibitory Concentrations of Vancomycin and Teicoplanin on Adherence of Staphylococci to Tissue Culture Plates

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**Bacterial adhesion is the first step in infection of medical devices. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the pathogens recovered most often. The effects of subinhibitory concentrations of vancomycin and teicoplanin on the adherence of eight clinical strains of *S. aureus* and eight strains of *S. epidermidis* to tissue culture plates in vitro were tested. The mean relative inhibitions of adherence at one-fourth and one-eighth the MIC were statistically different for teicoplanin and vancomycin. Slime production seemed not to be involved in adherence.**

Infection associated with the use of implanted biomaterials continues to be a significant source of morbidity and mortality. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the pathogens most frequently associated with implants (7, 8).

The adherence of bacteria to implanted material is important in the pathogenesis of infection (1). In prophylaxis of infection of implanted devices one must therefore consider the effect of antibiotics at this critical stage. Previous studies have shown that subinhibitory concentrations of antimicrobial agents can influence both positively and negatively the adherence of a variety of microorganisms (5, 11). With the increasing incidence of methicillin-resistant staphylococci which are also resistant to most antimicrobial agents, more use is made of glycopeptides such as teicoplanin and vancomycin in the prophylaxis of infection of implanted devices. This study was conducted to examine the effects of subinhibitory concentrations of glycopeptide antibiotics on the adherence of clinical isolates of *S. aureus* and *S. epidermidis*.

Eight strains of *S. aureus* and *S. epidermidis* were used which had been isolated from hospitalized patients and selected on the basis of their adherence properties as described previously (4). Vancomycin was obtained from Eli Lilly, and teicoplanin was obtained from Marion Merrell Dow.

MICs of vancomycin and teicoplanin for the bacterial isolates were determined with a modified dilution method using the conditions under which adherence was measured. A bacterial inoculum of $10^6$ CFU/ml was added to Mueller-Hinton broth containing a final concentration of antibiotic ranging from 128 to 0.025 mg/liter. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth at 37°C after overnight incubation in air.

To avoid the effect of subculture on adherence and slime production, aliquots of a primary culture were preserved at −70°C in polyethylene glycol–Mueller-Hinton broth. These aliquots were used to measure slime production and adherence in parallel. Aliquots were revived by overnight primary culture on solid media. Single colonies were then picked and grown overnight in glass tubes containing Mueller-Hinton broth. The decanted overnight culture was used to measure adherence, and the tubes were used to quantitate slime. Slime production was measured by the method of Davenport et al. (3). The glass tubes were allowed to dry, and the internal walls were washed with Gram's safranin solution. The amount of stained slime was macroscopically semiquantitated as 0 (absent), +, ++, or +++ compared with standard laboratory prestained tubes.

The overnight cultures were diluted to a final concentration of $10^6$ CFU/ml with Mueller-Hinton broth containing the antibiotic at final concentrations equal to 1/2 to 1/16 the MIC for the strain. Two hundred microliters of the organism-drug solution was placed into tissue culture plate wells (Nunc, Roskilde, Denmark) and allowed to adhere for 6 h at 37°C. Controls were performed in the absence of antibiotic.

Those organisms that failed to adhere and remained in the supernatant were removed by gentle aspiration of the wells. The tissue culture wells were then washed four times with phosphate-buffered saline (pH 7.2). Adhering organisms were fixed with ethanol and stained with crystal violet (Merck). Excess stain was rinsed off with running tap water, and plates were dried. Adherent bacterial films were measured with a microELISA Auto Reader (Dynatech Laboratories) by using a wavelength of 570 nm (2).

Adherence data were collected for eight strains of *S. aureus* and eight strains of *S. epidermidis* and three MICs (one-half, one-fourth, and one-eighth the MIC) of teicoplanin and vancomycin. These were replicated on two to three separate days and on four plates each day.

Data were averaged across plates, and these means were used in the subsequent analysis. These data were averaged for each MIC, day, and genus of organism. Relative adherence was analyzed as a percentage of the control level and was expressed as follows: (OD of treated well/OD of control well) × 100, where OD is the optical density. Relative inhibition of adherence was expressed as follows: [1(−(OD of treated well/OD of control well)] × 100.

An analysis of variance was performed on the adherence data at each MIC. The analysis of variance took account of the variation due to organism, day, and treatment, together with the interaction terms treatment-day and treatment-organism.

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TABLE 1. Effects of subinhibitory concentrations of vancomycin and teicoplanin on *S. epidermidis* and *S. aureus* relative adherence activities

| Species | Strain no. | Slime production | Drug[b] | MIC (μg/ml) | % Adherence value ± SD[a] at: | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1/2 MIC | 1/4 MIC | 1/8 MIC |
| *S. epidermidis* | 2 | 0 | V | 2 | 35.9 ± 33 | 103.5 ± 9.6 | 105.3 ± 7.7 |
| | | | T | 4 | 43.9 ± 7.3 | 64.3 ± 15.2 | 69.8 ± 19.3 |
| | 16 | +++ | V | 1 | 65.4 ± 25 | 94.9 ± 20.2 | 113.8 ± 29.5 |
| | | | T | 8 | 104.4 ± 16.4 | 94.6 ± 12.4 | 70.7 ± 14.2 |
| | 70 | 0 | V | 1 | 4.8 ± 1.4 | 18.5 ± 7.8 | 57.2 ± 13.4 |
| | | | T | 2 | 2.6 ± 1.5 | 3.6 ± 1.5 | 11.3 ± 4.1 |
| | 77 | + | V | 2 | 41.4 ± 19.3 | 79.4 ± 8.3 | 88.8 ± 7.5 |
| | | | T | 2 | 32.4 ± 13.6 | 46.4 ± 6.7 | 57.3 ± 8.3 |
| | 93 | + | V | 1 | 64.9 ± 16.7 | 98 ± 14.8 | 98.8 ± 13.8 |
| | | | T | 2 | 6.0 ± 2.0 | 10.7 ± 2.8 | 52 ± 30.3 |
| | 104 | 0 | V | 2 | 77 ± 12.1 | 88.4 ± 8.3 | 93.4 ± 4.9 |
| | | | T | 4 | 128.1 ± 10.9 | 110.8 ± 9.7 | 96.2 ± 6.8 |
| | 127 | +++ | V | 2 | 23.5 ± 7.3 | 88.4 ± 7.6 | 93.7 ± 10.1 |
| | | | T | 2 | 20.7 ± 6 | 30.0 ± 7.8 | 60.4 ± 9.1 |
| | 131 | + | V | 2 | 80.7 ± 8.9 | 100.2 ± 7.9 | 100.4 ± 11.1 |
| | | | T | 8 | 119.2 ± 12.2 | 109.9 ± 23.1 | 99.1 ± 11 |
| | | | V | | 49.2 ± 27.1 | 83.9 ± 27.5 | 93.9 ± 16.8 |
| | | | T | | 57.2 ± 51.8 | 58.8 ± 43 | 64.3 ± 27.5 |
| *S. aureus* | 3 | +++ | V | 1 | 53.5 ± 53.3 | 108.1 ± 26.7 | 105.5 ± 25.7 |
| | | | T | 0.5 | 27 ± 26.2 | 46.8 ± 30.9 | 59 ± 17.1 |
| | 47 | + | V | 2 | 7.4 ± 7.7 | 101.5 ± 18.2 | 111.6 ± 16 |
| | | | T | 2 | 3.7 ± 3.2 | 6.8 ± 2.8 | 67.7 ± 11.5 |
| | 71 | ++ | V | 2 | 9.7 ± 4.5 | 47.4 ± 31 | 110.6 ± 19.7 |
| | | | T | 1 | 5.9 ± 3 | 7.4 ± 3.1 | 58.6 ± 7.1 |
| | 80 | 0 | V | 1 | 20.1 ± 10.3 | 100.1 ± 19.2 | 95.7 ± 15.4 |
| | | | T | 0.5 | 16.2 ± 8.7 | 48.3 ± 39.6 | 83.2 ± 23.3 |
| | 96 | 0 | V | 2 | 43.5 ± 42 | 104.4 ± 4.5 | 93.6 ± 8.9 |
| | | | T | 0.5 | 3.7 ± 2.6 | 75.3 ± 25 | 104.7 ± 11 |
| | 159 | 0 | V | 2 | 4.4 ± 3.3 | 12.3 ± 4.4 | 105.5 ± 8.2 |
| | | | T | 1 | 3.8 ± 1.7 | 20.7 ± 7.2 | 73.8 ± 5.5 |
| | 183 | ++ | V | 1 | 10.5 ± 2.9 | 99.8 ± 11.5 | 96.1 ± 6.5 |
| | | | T | 1 | 7.8 ± 2.3 | 10.4 ± 3.1 | 53.9 ± 6.7 |
| | 192 | + | V | 1 | 9.6 ± 3.2 | 107.3 ± 15.7 | 99.3 ± 12.2 |
| | | | T | 0.5 | 4.4 ± 3.5 | 15.6 ± 10.6 | 80.1 ± 23.1 |
| | | | V | | 19.9 ± 18.4 | 85.1 ± 3.5 | 102.2 ± 7 |
| | | | T | | 9.1 ± 8.4 | 28.9 ± 25 | 72.6 ± 16.7 |

[a] Data are mean percent adherence values of treated cultures ± standard deviation compared with nontreated cultures (control).
[b] V, vancomycin; T, teicoplanin.

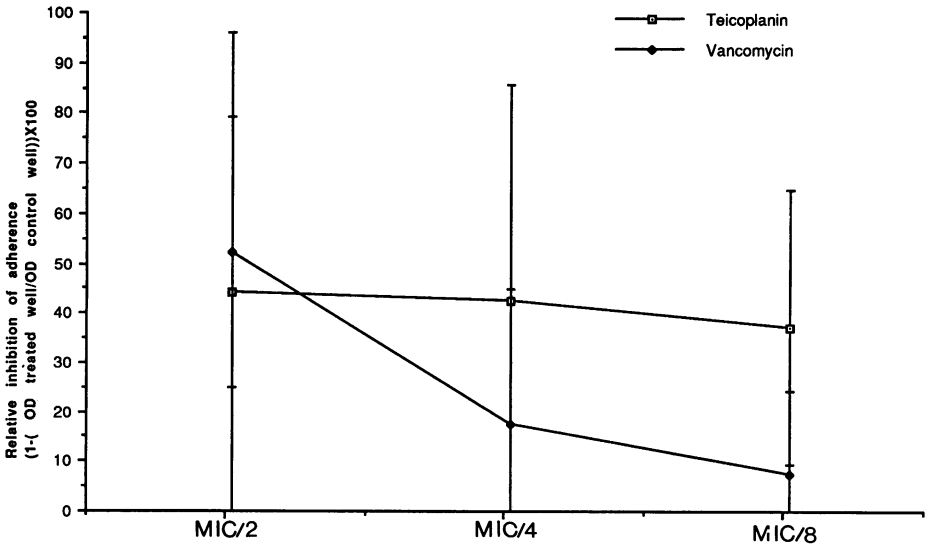


FIG. 1. Relative inhibition of adherence of eight strains of *S. epidermidis* by teicoplanin or vancomycin. MIC/2, one-half MIC; MIC/4, one-fourth MIC; MIC/8, one-eighth MIC.

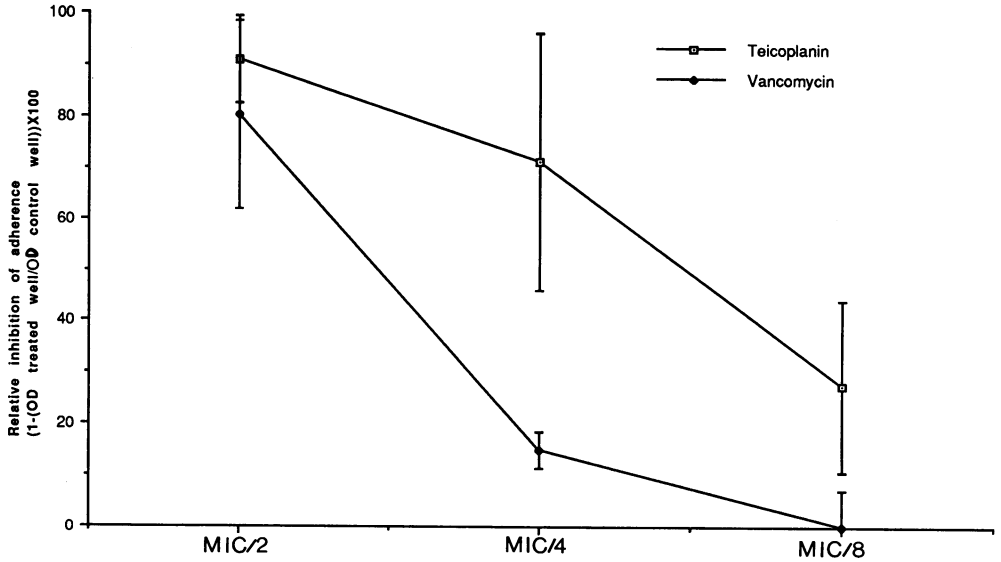


FIG. 2. Relative inhibition of adherence of eight strains of *S. aureus* by teicoplanin or vancomycin. MIC/2, one-half MIC; MIC/4, one-fourth MIC; MIC/8, one-eighth MIC.

Table 1 shows the percent means of adherence relative to the control at each MIC and by organism type for *S. aureus* and *S. epidermidis*. The mean relative inhibitions of adherence for teicoplanin at one-half, one-fourth, and one-eighth the MIC were 42.8, 41.2, and 35.7% for *S. epidermidis* and 90.9, 71.1, and 27.4% for *S. aureus*. For vancomycin, they were 50.8, 16.1, and 6.1% for *S. epidermidis* and 80.1, 14.9, and 0% for *S. aureus*. The differences were statistically significant for the one-fourth- and one-eighth-MIC values ($P < 0.001$) (Fig. 1 and 2).

At one-fourth the MIC, there was a difference in the teicoplanin percent inhibition of adherence relative to the control for *S. aureus* (71.1%) compared with *S. epidermidis* (41.2%). There was no such difference at one-eighth the MIC (14.9% versus 16.1%, respectively).

Numerous reports have described morphologic and ultrastructural alterations of staphylococci in the presence of subinhibitory concentrations of antibiotics (5, 6, 9, 10). Shibl (11) found that subinhibitory concentrations of clindamycin, lincomycin, erythromycin, and chloramphenicol decreased adherence of *S. aureus* to tissue culture plates, whereas vancomycin had no activity. Our results are in agreement with their results. All strains showed reduced adhesion at one-half the MIC of both antibiotics and at one-fourth the MIC of teicoplanin for *S. aureus* and *S. epidermidis* strains tested.

Although our results suggested a better activity of teicoplanin for *S. aureus* and *S. epidermidis*, further work is needed to confirm and clarify the relationship between the relative adherence-inhibiting properties of teicoplanin observed in our study and the mechanisms involved. Particular attention needs to be paid to the large variation in inhibition of adherence in *S. epidermidis* compared with the relatively uniform results for the eight strains of *S. aureus*. Enhancement of adherence was seen in three *S. epidermidis* isolates with teicoplanin. A similar increase was previously described with beta-lactams (11).

According to our results, the observed effects of antibiotics on adhesion are independent of slime production (Table 1). Thus, the production of a slimy matrix in which bacteria become embedded may be essential to establish a more firm and stable attachment once adherence has taken place.

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