The Effect of Vancomycin on the Structure of Vancomycin-Susceptible and -Resistant Enterococcus faecium Strains

Williamson et al. previously showed that a strain of Enterococcus faecium with low-level resistance to vancomycin grown in the presence of one-half the MIC of vancomycin showed bizarre cell forms with abnormal septa without apparent cell division (3). In the current work, 10 strains of E. faecium susceptible to vancomycin (MIC, <2 μg/ml) and 10 resistant strains (MIC, >400 μg/ml) which were also resistant to teicoplanin (MIC, >40 μg/ml) were DNA fingerprinted to exclude the possibility of strain duplication. Vancomycin at one-half the MIC (0.3 to 0.6 μg/ml for susceptible and 200 to 250 μg/ml for resistant strains) was added to 10-ml volumes of Trypticase soy broth (TSB) which were inoculated with 100 μl of a 1/10 dilution of the respective 24-h TSB cultures. The cultures were sampled periodically throughout the 24-h period of incubation for Gram staining. Two strains resistant to vancomycin, grown with vancomycin for 12 h, were washed and reincubated in TSB, and Gram stains were prepared hourly for 6 h.

Three vancomycin-susceptible and three vancomycin-resistant strains, from the above-described strains, were grown for 12 h with or without vancomycin and examined by electron microscopy by a technique previously described (2). Cell size was determined from photographs at final magnifications of ×20,000. The means and standard deviations were calculated and statistically analyzed.

On Gram stains, the 20 strains of E. faecium grown for 12 h in TSB were of normal appearance (data not shown). The appearances of all vancomycin-susceptible E. faecium strains exposed to one-half the MIC of vancomycin were comparable to that of the control. In contrast, all 10 vancomycin-resistant E. faecium strains exposed to one-half the MIC of vancomycin showed morphologies clearly distinct from that of the control. The majority of cells were rod shaped; numerous chains and large bizarre forms were also present.

In electron microscopy photographs, the mean lengths of cells for three vancomycin-resistant strains exposed to vancomycin were 2.05, 2.04, and 1.96 μm (long cells) and mean lengths of cells for three susceptible strains were 1.07, 1.24 and 1.4 μm (P < 0.001). The mean length of the controls, strains grown in TSB without vancomycin, was 1.16 μm. Most long cells resulting from exposure to vancomycin consisted of a chain of individual organisms with a transverse diameter.

FIG. 1. (A) Vancomycin-resistant E. faecium exposed to vancomycin. Bar = 1 μm. (B) Vancomycin-resistant E. faecium exposed to vancomycin. The inset shows a control cell grown in TSB alone. Bar = 1 μm.
almost twice the size of the control grown without vancomycin. These organisms were bound one to another by wide abnormal cross-walls (Fig. 1). Other large cells consisted of the same elements arranged in a variety of nonlinear patterns.

Subinhibitory concentrations of vancomycin seemed to block autolytic activity in vancomycin-resistant strains without affecting the vancomycin-susceptible organisms. When washed and incubated in TSB, the long cells separated, after 5 h of incubation, into cells of normal size, suggesting normal autolytic activity. Division without separation has been reported with Staphylococcus aureus exposed to subinhibitory concentration of penicillin (1). It appears that vancomycin inhibits the autolytic system of vancomycin-resistant E. faecium without affecting the process of division.

REFERENCES


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