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In Vitro 24-Hour Time-Kill Studies of Vancomycin and Linezolid in Combination versus Methicillin-Resistant *Staphylococcus aureus*▽

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Many clinicians are trying unique strategies, including vancomycin and linezolid in combination, for treatment of patients who do not respond to conventional therapy against methicillin (meticillin)-resistant *Staphylococcus aureus*. In our study, which illustrated in vitro activity only, no synergistic activity was seen when the two agents were combined. Conversely, antagonistic activity occurred in three of five strains when linezolid was added to vancomycin. Our results indicate that vancomycin and linezolid in combination should be avoided.

Methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) is a well-known nosocomial pathogen. There is considerable evidence showing that the incidence and possibly the virulence are rising (5, 8, 9, 10). Deep-seated infections are not responding as well to conventional vancomycin therapy (11, 15, 16). There have been case reports of not only vancomycin tolerance but also vancomycin resistance (3). This has an impact on the healthy population, with the growing incidence of community-acquired MRSA, and is of even more concern for the ever-growing number of elderly patients with numerous comorbid conditions, including hospitalized and nursing home patients (4, 6, 7, 17). Numerous alternative regimens are being tested in this era of multidrug-resistant organisms. These regimens include various lengths of treatment as well as combinations of antimicrobial agents. We have observed an increase in the use of linezolid and vancomycin together, with little evidence to support this practice. There is also the potential for overlap when one agent is switched to the other. Vancomycin is a bactericidal agent which inhibits bacterial cell wall synthesis, resulting in cell lysis. Linezolid is a bacteriostatic agent which binds the 50S ribosomal unit and inhibits protein synthesis. Few previous studies show the activity of these two antibiotics in combination. There have been approximately 20 strains of MRSA tested, and of these strains, several have shown no difference in activity while others suggest that linezolid and vancomycin may actually be antagonistic (2, 12, 14, 18). Our investigation was designed to consider additional MRSA isolates in a different geographic region and help provide more evidence about the interaction that vancomycin and linezolid have when used together.

In order to start this study, we first obtained five strains of MRSA isolates from blood cultures at Christiana Hospital and tested them to be sure they were different ribotypes. Next, the MICs for each strain were determined using the microdilution technique. The MICs were defined as the lowest concentration of an antimicrobial agent that prevented turbidity when assessed after 24 h of inoculation. MIC determination was repeated twice for consistency, and the average value for all three determinations was used as the final MIC. The MICs were 0.06 μg/ml for vancomycin in all isolates; 0.125 μg/ml for linezolid in isolates 1, 2, 4, and 5; and 0.25 μg/ml for isolate 3.

The time-kill study was performed with four concurrent tubes that were run over 24 h. All stock solutions were prepared in accordance with guidelines provided by the Clinical and Laboratory Standards Institute, formerly known as NCCLS (1). Each tube contained Mueller-Hinton broth, with an inoculum of 5×10^6 to 1×10^7 CFU/ml. Tube 1 was antibiotic free and served as the control. Tubes 2 and 3 had linezolid and vancomycin, respectively. Tube 4 contained both linezolid and vancomycin. All tubes were run with all five strains of MRSA separately. Surviving bacteria were counted at 0, 4, 8, and 24 h by subculturing 50-μl serial dilutions (10^{-1}, 10^{-2}, and 10^{-4}) of samples in normal saline solution on Mueller-Hinton plates. The above was done at one-fourth, one-half, and two times the MIC for each agent. Bactericidal effect was defined as $\geq 3 \log_{10}$ CFU/ml decrease in comparison with the level for the initial inoculum after 24 h of incubation. Synergy was defined as a decrease of $\geq 2 \log_{10}$ CFU/ml between the combination and the most active single agent. Antagonism was defined as an increase in the colony count of $\geq 2 \log_{10}$ CFU/ml with the combination in comparison with the count obtained with the most active single agent (13). This entire process was repeated for all five strains at one-fourth, one-half, and two times the MIC for each agent alone and in combination.

The final data were best delineated at two times the MIC. Figure 1A through E show the macrodilution time-kill curves for all five strains at two times the MIC. These show that for MRSA strains 2, 4, and 5, vancomycin was more effective when used alone than when linezolid was added. In strain number 1, there was no significant difference between each agent alone and combined. In strain number 3, vancomycin alone and linezolid alone were similar in activity. When the two agents were combined, they were less active than either agent alone, although this result was not significant enough to indicate an-

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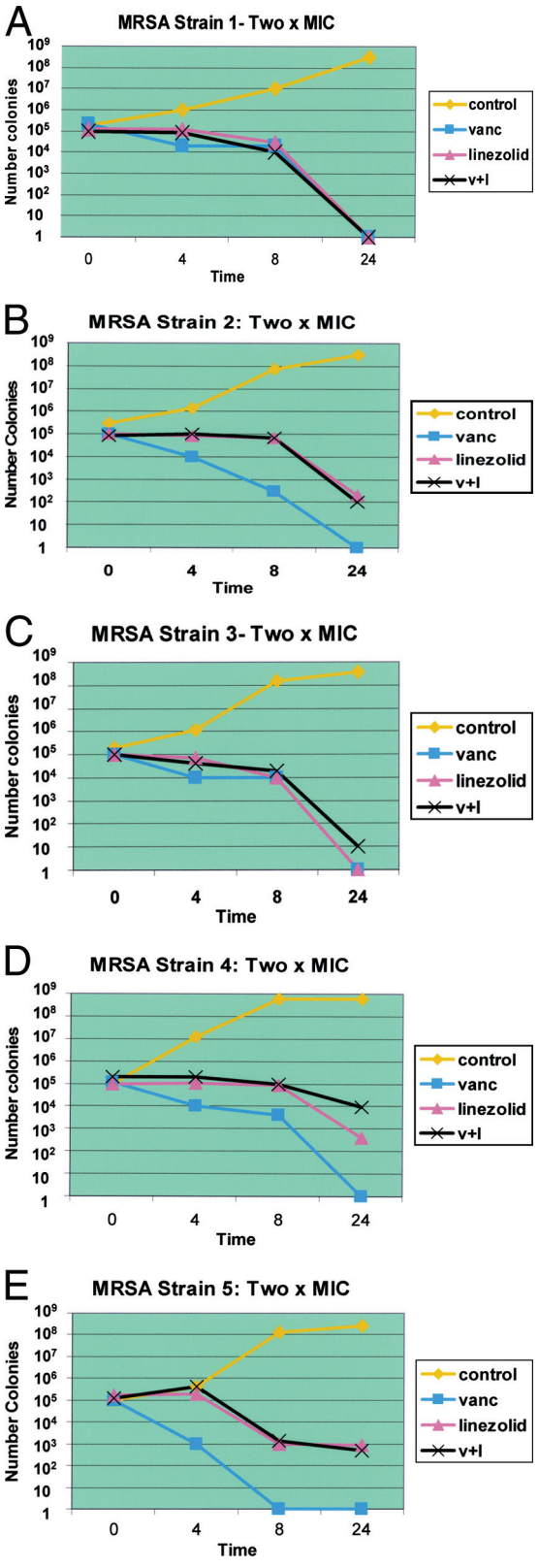

FIG. 1. Panels A through E graphically illustrate the results at two times the MIC for each bacterial strain, comparing the activity of vancomycin and linezolid together against that of each antibiotic alone as well as the results for the control group, which did not contain any antibiotics.

tagonism. No synergistic activity was seen in any of the five strains of MRSA. Three of the five strains exhibited antagonistic activity when linezolid was added to vancomycin. Two of the strains were equivocal. This data set indicates that combination therapy is of no benefit and that vancomycin and linezolid should not be used together for MRSA infections. These results suggest that special attention may need to be given to patients with illnesses such as chronic renal disease when one agent is switched to another since these antibiotics may have altered kinetics or a prolonged half-life. We hypothesize that this antagonism may be due to a reduced ability on the part of vancomycin to bind to cells exposed to linezolid, which is bacteriostatic and decreases protein synthesis.

These results do not account for tissue penetration and metabolism which alter the in vivo activity of these agents when used in combination. There needs to be more data both in vivo and in vitro to demonstrate the interaction between these two agents. These types of combinations as well as new agents will need to be studied as MRSA becomes more resistant and infections become more severe and harder to eradicate.

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