Simple Test of Synergy between Ampicillin and Vancomycin for Resistant Strains of Enterococcus faecium

MICHAEL GREEN,1,2* KAREN BARBADORA,3 AND ROBERT M. WADOWSKY3,4

Departments of Pediatrics,1 Surgery,2 and Pathology,3 Children’s Hospital of Pittsburgh, University of Pittsburgh School of Medicine, and Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh,4 Pittsburgh, Pennsylvania 15213

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The combination of ampicillin and vancomycin kills some but not all strains of ampicillin- and vancomycin-resistant Enterococcus faecium. We compared a simple test for synergy utilizing a commercially available microdilution susceptibility system with time-kill studies and determined acceptable breakpoints for this test for 20 strains of ampicillin- and vancomycin-resistant E. faecium. The combination of ampicillin and vancomycin was tested for synergy by time-kill, broth microdilution, and broth macrodilution procedures. Repeat testing of isolates by macro- and microdilution synergy methods yielded MICs that were within one twofold dilution of each other for both intra- and intertest comparisons. Synergy was always detected by time-kill studies when the MIC of ampicillin in the combination microdilution synergy screen was ≤8 μg/ml in the presence of vancomycin. No synergy was detected when the MIC was >16 μg/ml in the combination microdilution synergy screen. The determination of the synergy by the broth microdilution procedure appears to be simple, convenient, and accurate.

Vancomycin resistance among enterococci has recently been recognized (10). Strains exhibiting inducible, high levels of resistance to both vancomycin and teicoplanin are designated VanA (12). VanB strains express variable levels of resistance to vancomycin but usually remain susceptible to teicoplanin (3). Although Enterococcus faecium may be resistant to both ampicillin and vancomycin, synergy between these two antibiotics has been demonstrated in vitro (9, 12) and in a rabbit model of endocarditis (1). However, isolates resistant to the combination of ampicillin and vancomycin among ampicillin- and vancomycin-resistant Enterococcus faecium (AVREF) have been reported (2, 4–7), suggesting the necessity for in vitro evaluation of the ampicillin-vancomycin combination in all clinical cases in which its use is considered. The purpose of this study was to compare a simple test for synergy utilizing a commercially available microdilution susceptibility system (Sensititer) with time-kill studies and to determine acceptable breakpoints for this test in the assessment of combination ampicillin-vancomycin therapy in patients infected with AVREF.

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Twenty isolates of E. faecium resistant to ampicillin (MIC ≥16 μg/ml) and vancomycin (MIC ≥16 μg/ml) were evaluated in this study. Six isolates were recovered from liver transplant recipients at the Children’s Hospital of Pittsburgh (LT27, LT96, LT97, LT146, and LT161) or Presbyterian University Hospital (W16804), and 14 isolates were from various geographic sites (CK1, JK1, SF1, JB1, JB51, JB61, JB71, M1, MUSE, L91, H91, G91, BM4147, and NYMC).

The combination of ampicillin and vancomycin was tested for synergy by time-kill, broth microdilution synergy screen, and broth microdilution synergy screen methods. In order to induce vancomycin resistance, isolates were grown overnight in a subinhibitory concentration of vancomycin (8 μg/ml) prior to synergy testing. Brain heart infusion broth was used for the microdilution and time-kill studies. Microdilution tests were performed with cation-supplemented Mueller-Hinton broth and the Sensititer system (Radiometer, Copenhagen, Denmark). Each of the three methods was performed in triplicate on separate days for isolates LT146, LT161, BM4147, W61804, and NYMC. The remaining 15 isolates were used in a single time-kill study and broth microdilution synergy screen. Time-kill studies were performed with standardized inocula (13) and a combination of ampicillin (16 μg/ml) and vancomycin (8 μg/ml). These concentrations were chosen because they represented achievable levels of both antibiotics in blood but were less than the MICs for all tested organisms. Viable concentrations were determined at 0, 6, and 24 h. Synergy in time-kill studies was defined as a ≥100-fold increase in killing at 24 h compared with that of the most active single agent. A static effect was declared when (i) the combination of antibiotics resulted in a horizontal time-kill curve and (ii) the final concentration in this culture was at least 2 log10 CFU/ml less than the final concentration in the culture with the single most active agent.

Macrodilution and microdilution MICs were determined with overnight inocula adjusted with sterile deionized water to a density equal to a McFarland 0.5 standard (11). For macrodilution synergy testing, the suspensions were further diluted 1:200 in brain heart infusion broth containing both vancomycin (8 μg/ml) and twofold serial dilutions of ampicillin (range, 1 to 256 μg/ml). For microdilution synergy testing, a 10-μl aliquot of each adjusted suspension was transferred to 10 ml of cation-supplemented Mueller-Hinton broth containing vancomycin (8 μg/ml). The initial inoculum was approximately 5 × 10^8 CFU/ml, as confirmed by nephelometry. The suspension was inoculated into antibiotic panels according to the manufacturer’s instructions. The range of ampicillin used in these microdilution panels was 0.12 to 16 μg/ml. MICs were visually determined at 24 h of incubation for both methods. Repeat testing of isolates BM4147, W61804, NYMC, LT161,
and LT146 by macro- and microdilution synergy screens yielded MICs that were within one twofold dilution of each other for both intra- and intertest comparisons. Agreement on the presence or absence of synergy was seen with each isolate in each of the triplicate time-kill studies.

The comparability of time-kill and microdilution synergy testing was further assessed with all 20 study strains (Table 1). For all 11 strains for which synergy was found in the time-kill studies, the ampicillin MICs in the microdilution synergy screen were \( \leq 8 \) \( \mu \)g/ml. In contrast, for all six strains for which synergy was not found by time-kill studies, the ampicillin MICs in the microdilution synergy screen were \( > 16 \) \( \mu \)g/ml. For three of the strains, the MIC of ampicillin dropped from 32 \( \mu \)g/ml in the absence of vancomycin to 16 \( \mu \)g/ml in the presence of vancomycin. Although this twofold change by itself is not considered significant, it and the finding that 16 \( \mu \)g of ampicillin per ml and 8 \( \mu \)g of vancomycin per ml yielded a static effect in the time-kill studies suggest that this combination may provide some benefit in treating infections caused by these strains.

The increasing frequency of resistance to both vancomycin and ampicillin in \textit{E. faecium} presents an important management dilemma to the clinician. The expected licensure of teicoplanin offers a therapeutic alternative for those isolates expressing the VanB phenotype. However, teicoplanin resistance has developed in vivo in a VanB \textit{E. faecium} isolate (8), potentially limiting the utility of this new agent. The demonstration of synergy in vitro and in vivo of the combination of ampicillin and vancomycin against some but not all isolates of AVREF offers an alternative treatment regimen. However, evaluation for ampicillin-vancomycin synergy against clinical isolates is required.

Synergy is traditionally evaluated by either the time-kill or checkerboard technique. We chose the former, since previous published evaluations of the synergy of ampicillin and vancomycin against ampicillin- and vancomycin-resistant enterococci also utilized this test (9, 12). Performance of either of these two methods is cumbersome and time-consuming, and neither is widely available in clinical microbiology laboratories. However, the microdilution synergy test is a rapid and easy method to screen for ampicillin-vancomycin synergy against \textit{E. faecium}. Clinical laboratories can readily adapt commercial microdilution sensitivity systems for use in synergy testing. An MIC of ampicillin of \( \leq 8 \) \( \mu \)g/ml for the combination of ampicillin and vancomycin correlated with the presence of synergy as determined by the time-kill method and appears to represent an appropriate breakpoint for this test.

Excellent agreement was found among and within time-kill test, macrodilution synergy screen, and microdilution synergy screen results. The only exceptions to this occurred with isolates for which the MIC of ampicillin in the presence of vancomycin was 16 \( \mu \)g/ml, the same concentration of ampicillin with which the time-kill study was performed. In these cases, the combination of drugs resulted in a static effect, which is in contrast to the lack of inhibition found with all single antimicrobial agents tested. Thus, in patients for whom the final MIC is 16 \( \mu \)g/ml, inhibition of growth could be achieved; additional testing with time-kill studies may be warranted for patients for whom no alternative therapy is available. However, it may be difficult clinically to maintain adequate levels of ampicillin to take advantage of this effect.

The results of this study identified a variable degree of drop in ampicillin MICs in the presence of vancomycin among isolates against which synergy was demonstrated in time-kill studies. Although the typical decrease ranged from 100- to 1,000-fold, in several isolates only a 4- to 8-fold decrease was noted. The apparent mechanism of ampicillin-vancomycin synergy has been identified as the substitution of two high-molecular-weight penicillin-binding proteins for the low-molecular-weight PBP 5 as the essential target in cell wall synthesis (6). Two mechanisms have been reported to explain the loss of synergy. The first involves a decrease in the production of these high-molecular-weight penicillin-binding proteins, while the second is due to an unknown change on one or more of the conjugative plasmids responsible for glycopeptide resistance (6). The variation in the degree of drop in ampicillin MIC may be explained by an incomplete expression of either of these two resistance mechanisms.

Although synergy does not necessarily equate with bacteriocidal activity or with clinical outcome, the ampicillin-vancomycin combination offers a potential therapeutic option for patients for whom alternative therapies are not available. Accordingly, we recommend the rapid microdilution synergy screen as a simple, convenient, and accurate test of synergy between ampicillin and vancomycin for clinical isolates of AVREF. Our results suggest that synergy of the combination of ampicillin and vancomycin against strains of AVREF will be demonstrated in time-kill studies if an ampicillin MIC of \( \leq 8 \) \( \mu \)g/ml is obtained in the microdilution synergy screen. The use of strains characterized in this study should allow for appropriate quality control of this screening procedure.

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