No. 288

Lack of Synergy With Six Blood Isolates of MRSA (Vancomycin MIC of 2) Tested with Combinations of Vancomycin + Gentamicin, Vancomycin + Rifampin and Vancomycin + Cefazolin

Virginia Long,[3] Paul O'Keefe, [1,3] Paul Schreckenberger, [2,3]

Departments of [1]Medicine, [2]Pathology, and [3]Infectious Disease and Immunology Research Institute, Stritch School of Medicine., Loyola University Chicago

Paul C. Schreckenberger, Ph.D.

2160 S. First Ave. Maywood, Illinois 60153

Email: Pschrecken@lumc.edu

Phone: 1-708-216-5682

ABSTRACT

Background: Vancomycin (V) is the mainstay for treatment of infections caused by MRSA. Yet failures of therapy with V are common. Current guidelines recommend V alone for treatment of most serious infections. Exceptions include prosthetic valve endocarditis and in some instances of osteomyelitis and CNS infections where combinations of antibiotics are suggested.

Methods: We used timed kill-curves to test antibiotic combinations used in our center against six isolates of MRSA. These six with V MICs of 2 confirmed by Microscan and E-test were chosen from 15 blood isolates of MRSA saved over three years. We used V and gentamicin (G), V and rifampin (R), two combinations used to treat MRSA, and V and cefazolin (C), a combination recommended in recent publications. Colonies were counted in duplicate at 0, 4, 8, 12 and 24 hour time points. Determinations of synergy, indifference and antagonism were made at the 24 hour time point. Standard definitions requiring 2 log differences in cfu/mL were used.

Results:

Combination	Syngery	Indifferent	Antagonistic
Van + Gent	0	6	0
Van + Rifampin	0	5	1
Van + Cefazolin	0	6	0

Antibiotic synergy was not demonstrated with any of the combinations tested. In all but one experiment, the combination was "indifferent", however V + G was more active than V alone for three strains. Although "antagonistic" in only one, V+ R was less active than either drug alone for four of the isolates. Killing with V + C paralleled killing with V alone in all six strains.

Conclusion: We have shown that combining G, R or C with V against six bloodstream isolates of MRSA with V MIC of 2 is not synergistic *in vitro*. These results did show enhanced killing with V+G in three strains and with V+R in one strain. Killing with V + R was slightly worse in three and significantly worse in one of the isolates. These results, although not showing synergy, may support the use of V + G, and, although not showing antagonism, do not support use of V + R.

INTRODUCTION

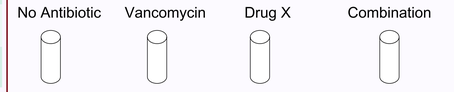
Multiple antibiotics are used in various combinations to treat MRSA, such as daptomycin, rifampin, linezolid, vancomycin and β-lactams. A survey of over 400 Infectious Disease Consultants (ICDs) revealed that in the case of persistent MRSA bacteremia with vancomycin MIC 2μg/mL, 72% of the IDCs would continue vancomycin but add an additional drug, typically rifampin or gentamicin (1). The *in vitro* pharmacodynamics of vancomycin and cefazolin against MRSA were studied by Hagihara et al (2). Time-kill studies demonstrated that combination therapy significantly reduced the bacterial concentration of MRSA when compared to vancomycin alone after 12 and 72 hours of incubation.

MATERIALS AND METHODS

Bacteria Strains: The six isolates used were chosen from 180 *S. aureus* isolates, collected over 3 years by the clinical microbiology laboratory at LUMC and stored in a -80°C freezer. The strains chosen for further study were MRSA blood isolates, with MICs of 2 confirmed by both Microscan and E-test.

Antibiotics: The antibiotics and concentrations used were vancomycin (10μg/mL), gentamicin (5μg/mL), rifampin (1μg/mL), and cefazolin (30μg/mL). Stock powders of the antibiotics were obtained from SIGMA-ALDRICH (St. Louis, MO) and dissolved in sterilized deionized water (vancomycin, gentamicin, cefazolin) or dimethyl sulfoxide (DMSO) (rifampin). The combinations used were vancomycin + gentamicin, vancomycin + rifampin, and vancomycin + cefazolin.

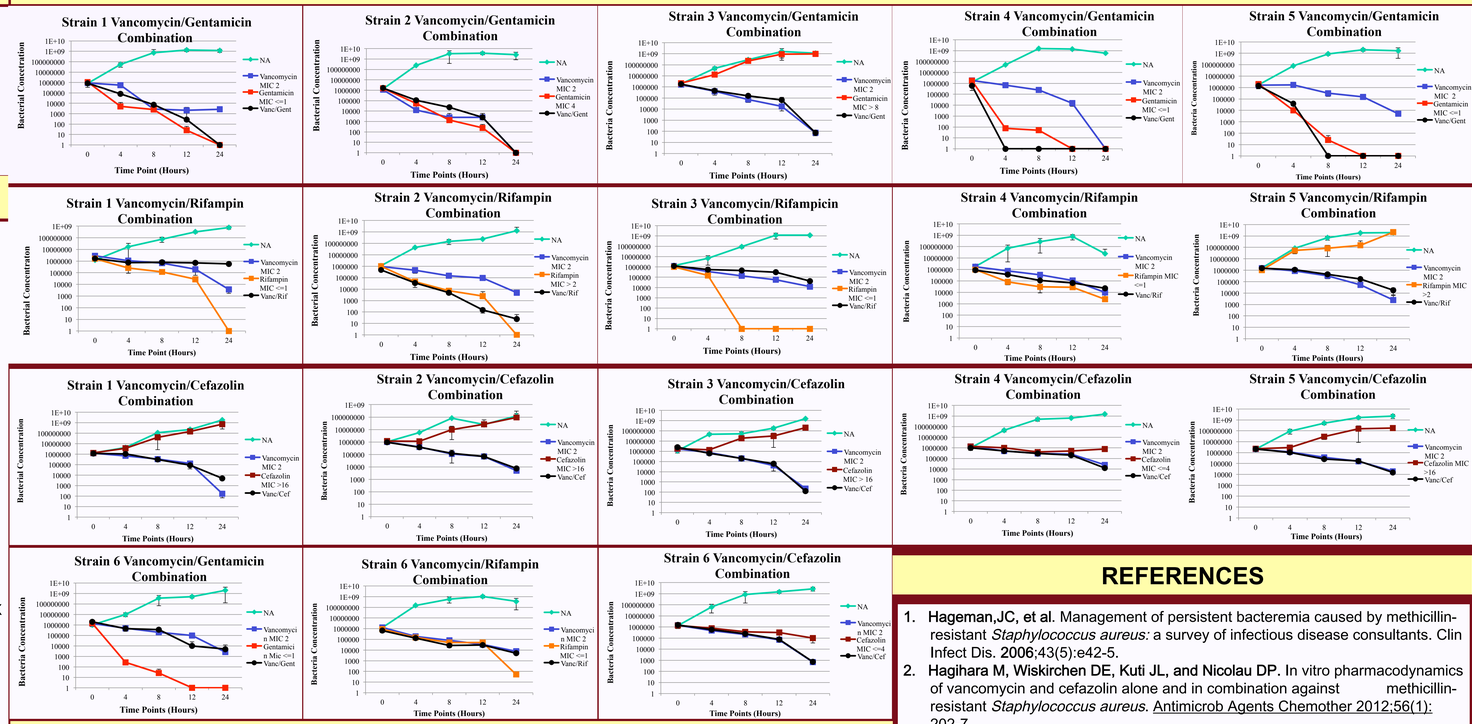
Time Kill Curves: Tube composition for each time-kill experiment is shown below:



The no antibiotic tube (NA) served as the control for each experiment, and as a growth curve for each strain. Drug X was gentamicin, rifampin, or cefazolin and 5μLof gentamicin stock, 2.5μL of rifampin stock, and 15μL of cefazolin stock were used to achieve the desired concentrations in each tube. Colony counts were performed from each tube at the following time points: 0, 4, 8, 12, 24 hrs.

Definitions: A combination was considered synergistic when at least a 2 $\log_{10}$ decline in CFU/mL was achieved at 24 hours by the drug combination compared to the most active single drug. Indifference of a combination was defined as a <2 $\log_{10}$ change in CFU/mL compared to the individual drugs at 24 hours. A combination was considered antagonistic when a 2 $\log_{10}$ increase in CFU/mL was achieved by the drug combination compared to both of the drugs individually at 24 hours. (3, 4)

RESULTS



DISCUSSION/CONCLUSION

Our results show that combining gentamicin, rifampin or cefazolin with vancomycin against six strains of MRSA with vancomycin MIC of 2 μg/mL is not synergistic *in vitro*. Enhanced killing with vancomycin + gentamicin was shown in three strains, and with vancomycin + rifampin in one strain. Killing with vancomycin + rifampin was worse than both antibiotics alone in four strains, and achieved antagonism in one of those strains. These results, although not demonstrating synergy may support the use vancomycin + gentamicin, but do not support the use of vancomycin + rifampin or vancomycin + cefazolin for treatment of MRSA infections.

REFERENCES

1. Hageman,JC, et al. Management of persistent bacteremia caused by methicillin-resistant *Staphylococcus aureus:* a survey of infectious disease consultants. Clin Infect Dis. 2006;43(5):e42-5.
2. Hagihara M, Wiskirchen DE, Kuti JL, and Nicolau DP. In vitro pharmacodynamics of vancomycin and cefazolin alone and in combination against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2012;56(1): 202-7.
3. Hackbarth, C. J., Chambers, H.F. and Sande, M. A. Serum bactericidal activity of rifampin in combination with other antimicrobial agents against *Staphylococcus aureus*. Antimicrob Agents Chemother. 1986;29(4):611-3.
4. Rochon-Edouard, S., Pestel-Caron, M., Lemeland, J.F., and Caron, F. In vitro synergistic effects of double and triple combinations of β-lactams, vancomycin, and netilmicin against methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother. 2000;44(11):3055-60.