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Bactericidal Action of Nafcillin, Vancomycin, and Three Cephalosporins Against Nafcillin-Susceptible and Nafcillin-Resistant Coagulase-Negative Staphylococci

Joyce J. Mordenti, Richard H. Davis, Claudia J. Lammel, and Geo. F. Brooks

Coagulase-negative staphylococci (*S. epidermidis*, 43 strains; *S. warneri*, 16 strains; *S. haemolyticus*, five strains; and others, four strains) were tested by the agar dilution method for nafcillin susceptibility: 53 were susceptible with a minimal inhibitory concentration (MIC) of ≤ 2 $\mu\text{g/ml}$; four were of indeterminate susceptibility, MIC = 4–16 $\mu\text{g/ml}$; and 11 were resistant, MIC ≥ 32 $\mu\text{g/ml}$. The bactericidal activities from 0 to 24 hr for nafcillin, vancomycin, cephalothin, cefazolin, and cefamandole, each at 16 $\mu\text{g/ml}$ in broth, were determined for all the isolates. The data indicate that a nafcillin agar dilution susceptibility test result of resistance does not consistently predict lack of killing activity by the cephalosporins. It is likely that each cephalosporin would have to be tested against individual coagulase-negative staphylococci in order to determine a suitable therapeutic or prophylactic cephalosporin, if a cephalosporin were to be used. Vancomycin was bactericidal for all the nafcillin-resistant coagulase-negative organisms tested.

INTRODUCTION

This study was undertaken to compare the differential bactericidal action of nafcillin, vancomycin, cephalothin, cefazolin, and cefamandole against nafcillin-susceptible and nafcillin-resistant coagulase-negative staphylococci. The methods of comparative analyses used in the study quantified static [minimal inhibitory concentration (MIC) and extent of kill] as well as kinetic (rate of kill) indices for all five drugs to study possible advantages for cephalosporins compared with the other drugs.

MATERIALS AND METHODS

Bacteria

Sixty-eight coagulase-negative staphylococci were obtained from patients' specimens submitted for culture. The 68 isolates included 43 strains of *S. epidermidis*, 16 strains of *S. warneri*, five strains of *S. haemolyticus*, and one strain each of *S. capitis*, *S.*

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hyicus, *S. sciuri*, and *S. simulans*. (The one strain of *S. hyicus* was coagulase-negative). These isolates were recovered from blood, cerebrospinal fluid, orthopedic wounds, pleural fluid, peritoneal fluid, trachea, or intravenous catheter tip. Each organism was isolated in pure culture or was the predominant organism in the culture. The organisms were considered to be potentially meaningful in terms of clinical infection; however, patient histories were not reviewed to determine if the isolates were truly causes of infection.

Antimicrobial Agents

Nafcillin, vancomycin, cephalothin, cefazolin, and cefamandole standard powders of known potency were obtained from the manufacturers.

Minimal Inhibitory Concentrations

The MICs for the five drugs were determined by agar dilution using methods of the National Committee on Clinical Laboratory Standards (1983), except that MIC end-points were read after 24 and 48 hr incubation at 35°C. The 48 hr incubation at 35°C was used to detect heteroresistance (Thornsberry et al., 1973). The 48-hr endpoints were used for the final data tabulations. *Streptococcus faecalis* ATCC 29212 was used as the control strain.

Bactericidal Assays

The bactericidal action of each antibiotic against the 68 organisms was measured in brain heart infusion broth with drug concentrations of 16 µg/ml. The concentration was chosen because it represented clinically achievable serum concentrations for each antibiotic. A drug-free growth control was included for each organism. Inocula, which had final concentrations of 1×10^6 CFU/ml, were made from overnight growth of the organisms on blood agar plates—an acceptable procedure for staphylococci (Baker et al., 1983). Test cultures were incubated at 37°C, and 0.1 ml samples were taken, diluted, and plated on Mueller–Hinton agar at 0, 2, 4, 6, 8, and 24 hr. Colonies formed by the surviving bacteria were counted after 48 hr incubation at 37°C. Data from a total of 408 bactericidal and control assays are reported in this study.

Data Analysis

The agar dilution nafcillin MIC (micrograms per milliliter) data were used to group organisms as susceptible ($\text{MIC} \leq 2 \mu\text{g/ml}$), indeterminant ($4 \leq \text{MIC} \leq 16 \mu\text{g/ml}$), and resistant ($\text{MIC} \geq 32 \mu\text{g/ml}$).

The rate of killing for each antibiotic was assessed by linear regression analysis on the $\log_{10}$ CFU/ml versus time data for the 2, 4, and 6 hr time points. These time points were chosen because the slopes of the time-kill data lines from 2 to 6 hr represented the initial rate of killing for each antibiotic.

The extent of bactericidal action was assessed by comparing the number of viable organisms remaining after 24 hr incubation. A 99.9% reduction ($3 \log_{10}$ decrease) at 24 hr was interpreted as bactericidal; a <99.9% reduction in CFU/ml at 24 hr was considered bacteriostatic; an increase in CFU/ml at 24 hr compared with zero time was considered to be noninhibition (e.g., growth).

Data analysis was performed using the National Institutes of Health-sponsored PROPHET computer network (Castleman et al., 1975). For small sample sizes ($n \leq 50$), the Wilk–Shapiro test for normality was used to test the hypothesis that a sample

came from a population that was normally distributed. For larger sample sizes ($n > 50$), the D'Agostino test for normality was used (NIH Publication No. 80-2169, 1980). The Kruskal-Wallis test was used to compare data that were not normally distributed. Multiple pairwise comparisons were performed whenever a significant difference was detected with the Kruskal-Wallis test, using a procedure that paralleled the methodology of the Neuman-Kuels test, except that sample rank sums were used instead of means (NIH Publication No. 80-2169, 1980).

A p value of <0.05 was used for overall significance, and a p value of <0.01 was used for detecting differences in pairwise comparisons.

RESULTS

Antibiotic Inhibitory Concentrations

The agar dilution susceptibility tests revealed 53 nafcillin-susceptible organisms, four of indeterminate susceptibility, and 11 resistant organisms (Figure 1). The resistant organisms were predominantly *S. epidermidis*, but included *S. warneri* and *S. haemolyticus*. There was no significant differential susceptibility pattern between species or for isolates from different anatomic sites. Organisms that were susceptible

FIGURE 1. Histogram of susceptibility test results. S indicates susceptible, I indicates indeterminate (used only for nafcillin), and R indicates resistant. All the isolates were susceptible to vancomycin. The isolates were considered to be cephalosporin resistant if they grew at concentrations of $\geq 16 \mu\text{g/ml}$.

TABLE 1. Rate Constants (K) of Decreasing Colony Counts as a Function of Nafcillin Susceptibility for 68 Clinical Isolates of Coagulase-Negative Staphylococci

Drug	K (hr ⁻¹)			Comparison of killing rates for nafcillin S, I, R, groups ^a
	Nafcillin (S) susceptible MIC ≤ 2 (n = 53)	Nafcillin (I) indeterminate 4 ≤ MIC ≤ 16 (n = 4)	Nafcillin (R) resistant MIC ≥ 32 (n = 11)	
Nafcillin	-0.476 ^b	-0.369	-0.127	p = 0.001
Vancomycin	-0.407	-0.347	-0.335	p = 0.325
Cephalothin	-0.423	-0.396	-0.393	p = 0.437
Cefazolin	-0.463	-0.256	-0.281	p = 0.011
Cefamandole	-0.448	-0.372	-0.456	p = 0.721
Control	+0.852	+1.073	+0.939	p = 0.231

^aKruskal-Wallis test.

^bNegative values indicate decreasing CFU/ml, and positive values indicate growth.

to nafcillin were susceptible to the other antibiotics; some nafcillin-resistant organisms, however, were susceptible to some of the cephalosporins. All of the isolates were susceptible to vancomycin.

Bactericidal Assay Data

The plots of log₁₀ CFU/ml versus time were approximately linear from 2 to 6 hr. An equation of the form $y = mx + b$ was used to describe the data as follows:

$$\log_{10} (\text{CFU/ml})_t = \log_{10} (\text{CFU/ml})_{t^0} - K (t^0 - t),$$

where the subscripts t and t^0 refer to the time of the CFU/ml determinations, K (the rate constant of antibiotic killing) is the slope of the log₁₀ CFU/ml versus time plot, and $(t^0 - t)$ is the elapsed time in hours between the CFU/ml determinations. Taking the antilog of both sides, the equation reduces to

$$(\text{CFU/ml})_t = (\text{CFU/ml})_{t^0} e^{-2.303 K (t^0 - t)},$$

where 2.303 is the factor needed for converting the log₁₀ equation to natural logarithms.

The rate constants for bactericidal action (K) for nafcillin-susceptible, -indeterminate, and -resistant organisms were not normally distributed; therefore, median values are shown in Table 1. The Kruskal-Wallis test was used to compare the rate of antibiotic killing between treatment groups within the staphylococcal nafcillin-susceptibility subclasses (columns of Table 1) and to compare the rate of antibiotic killing between nafcillin-susceptibility subclasses within each treatment trial (rows of Table 1). The rate constants for antibiotic killing of nafcillin-susceptible strains were comparable for all the antibiotics, with only the controls being significantly different. Both nafcillin and cefazolin had low rate constants for nafcillin-resistant strains, whereas the rate constants for vancomycin, cephalothin, and cefamandole changed very little; only the controls were significantly different. When comparisons were made of the rates of antibiotic killing within treatment groups, only nafcillin

FIGURE 2. Cumulative results of 24-hr CFU/ml remaining in the bactericidal assays for nafcillin-susceptible (top panel), nafcillin-indeterminate (middle panel), and nafcillin-resistant (bottom panel) isolates. The data are shown as the mean of the log₁₀ CFU/ml $\pm$ SD.

and cefazolin showed significant decreases in killing rates between the nafcillin-susceptible and nafcillin-resistant strains.

Figure 2 shows the extent of bactericidal action at 24 hr. A one-way analysis of variance was used to compare the number of viable bacteria remaining at 24 hr in each nafcillin-susceptibility subclass. For nafcillin-susceptible and -indeterminate organisms, the decrease in colony counts at 24 hr was similar for all five drugs and significantly different than the controls. For nafcillin-resistant organisms, vancomycin showed significantly greater bactericidal activity when compared with the other four drugs; furthermore, all the drugs except nafcillin were significantly different from the controls. On an individual basis, the 24 hr bactericidal actions of the drugs were vancomycin > cephalosporins > nafcillin.

An agar dilution susceptibility test result of nafcillin-susceptible predicted that nafcillin and the three cephalosporins were bactericidal for the majority of strains and bacteriostatic for the remaining strains. The results for the 15 strains of indeterminate or resistant susceptibility to nafcillin were less clear cut, with the four drugs being bactericidal, bacteriostatic, or noninhibitory (Table 2).

The MICs indicated all 68 strains were susceptible to vancomycin. Vancomycin was bactericidal for 45 of the 53 strains that were susceptible to nafcillin and bacteriostatic for the remaining eight strains—a result similar to that of the other four

TABLE 2. Extent of Antibiotic Bactericidal Action on the Coagulase-Negative Staphylococci as a Function of Nafcillin Susceptibility^a

	Drug tested in bactericidal assay				
	Nafcillin	Vancomycin	Cephalothin	Cefazolin	Cefamandole
Nafcillin susceptible (n = 53)					
Bactericidal	45	45	50	46	45
Bacteriostatic	8	8	3	7	8
Not inhibited	0	0	0	0	0
Nafcillin indeterminate (n = 4)					
Bactericidal	1	3	3	2	3
Bacteriostatic	3	1	0	1	0
Not inhibited	0	0	1	1	1
Nafcillin resistant (n = 11)					
Bactericidal	1 ^b	11	5	4	6
Bacteriostatic	4	0	2	2	1
Not inhibited	6	0	4	5	4

^aDetermined by 48-hr agar dilution minimal inhibitory concentration (MIC) tests.

^bThe nafcillin agar dilution MIC for this strain was 32 µg/ml; the broth dilution MIC was 2 µg/ml. The strain also exhibited heteroresistance.

drugs. Vancomycin was bactericidal for 14 of the remaining 15 strains and was bacteriostatic against one strain of indeterminate nafcillin susceptibility (Table 2).

DISCUSSION

In vitro susceptibility testing of *Staphylococcus epidermidis*-group organisms and *Staphylococcus aureus*, for the semisynthetic penicillinase-resistant penicillins, is subject to major difficulties in interpretation of results. Much more data are available for *S. aureus* than for the other species of staphylococci (Barry and Badal, 1977; McDougal and Thornsberry, 1984; Sabath, 1977; Thornsberry et al., 1973; Thornsberry and McDougal, 1983). *Staphylococcus aureus*, which are clearly resistant to nafcillin (or oxacillin or methicillin), also show resistance to cephalosporins by in vitro bactericidal assays and in the clinical setting; it is, however, not clear from published data that the same statements can be made for the coagulase-negative staphylococci (Ein et al., 1979; Frongillo et al., 1984; Hansen, 1983; John and McNeill, 1980; Lavender et al., 1978).

The isolates used in this study were not preselected for known nafcillin resistance and were a representative sample of typical patient isolates. The anatomic sites and species distribution of the isolates were representative of coagulase-negative organisms in general (Gill, 1983; Marsik and Brake, 1982; Price and Fournoy, 1982; Sewell et al., 1982). *S. epidermidis* and *S. haemolyticus* have been reported to show a wider spectrum of antibiotic resistance than other species in the group (Gill, 1983; Marsik and Brake, 1982; Price and Fournoy, 1982). We found no obvious differences in nafcillin susceptibility by species, but tested relatively few strains of species other than *S. epidermidis* and *S. warneri*.

The addition of 2%–5% NaCl to the agar or broth has been advocated for susceptibility testing of *S. aureus*. The utility of added NaCl in susceptibility testing of coagulase-negative staphylococci is less clear and has not been standardized for bactericidal assays of these bacteria. Therefore, we chose not to add NaCl to our assay system.

Cephalothin and cefamandole killed nafcillin-resistant organisms as swiftly as they killed nafcillin-susceptible strains. A likely explanation for this observation is that several of the nafcillin-resistant strains were susceptible to cephalothin and cefamandole. Comparison of the agar dilution results indicated this to be true. Similarly, comparison of the 24-hr bactericidal assay results indicated that all three cephalosporins were bactericidal for some of the nafcillin-resistant strains. This result could not be predicted by the nafcillin susceptibility test result, but did correlate with the specific cephalosporin susceptibility test result.

Cephalothin cumulative MICs were slightly lower than cefazolin and cefamandole cumulative MICs. Siebert et al. (1979) noted differences in cumulative MICs and minimal bactericidal concentrations against methicillin-resistant *S. epidermidis*, with cefazolin less active than cephalothin. We found that cefazolin killed the nafcillin-resistant strains more slowly than cephalothin or cefamandole when the drug concentrations were 16 µg/ml. The results of the 24-hr bactericidal assays, however, were not significantly different for cefazolin compared with cephalothin or cefamandole. We conclude that the bactericidal activities of the three cephalosporins were comparable even though the rates of killing were different. To choose one of the three cephalosporins over the others based on putative differences in potential bactericidal activity seems unwarranted.

The primary objective of this study was to determine whether or not in vitro susceptibility tests of coagulase-negative staphylococci for nafcillin accurately reflect concomitant resistance to cephalothin, cefazolin, and cefamandole. Nafcillin resistance apparently does not uniformly predict cephalosporin resistance. A secondary objective was to determine if the three cephalosporins showed differential activity against the nafcillin-resistant strains. Although cefazolin killed the organisms more slowly than cephalothin or cefamandole, each at 16 µg/ml, there was no difference in the bactericidal action at 24 hr. The data from the agar dilution MIC tests and the bactericidal assays indicated that the objectives were met, in terms of in vitro results.

Extrapolation of the results to the in vivo setting would require a parallel trial in animals, but we are not convinced such a study should be done. Animal model experiments, although sometimes yielding useful information for human infection, often produce data that are difficult to apply to human *S. epidermidis* infections (Archer et al., 1980). Differences in serum protein binding of drug, pharmacokinetics of drug, and methods used to initiate infection in animal models introduce complexities that are sometimes difficult to interpret. Furthermore, not all coagulase-negative organisms have a similar virulence in animals. Baddour et al. (1984) consistently produced experimental endocarditis in rats with *S. epidermidis*, whereas they were rarely successful with *S. hominis*.

A clinical trial with cephalosporin therapy of selected patients infected with nafcillin-resistant coagulase-negative staphylococci could be done, but may not be warranted given the availability of acceptable alternative therapy. If a cephalosporin were to be used, each isolate would need to be tested for susceptibility to the lethal action of the cephalosporins in order to select the most appropriate drug.

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