Vancomycin Treatment Failure Associated with Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* in a Patient with Endocarditis and in the Rabbit Model of Endocarditis

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Heterogeneous resistance to vancomycin is thought to precede emergence of intermediate susceptibility to vancomycin in *Staphylococcus aureus*, but the clinical significance of heterogeneous resistance is unknown. Paired *S. aureus* isolates from a patient with endocarditis who relapsed after vancomycin treatment were tested for heterogeneous resistance to vancomycin. The pretreatment and the relapse clinical isolates (strains SF1 and SF2, respectively) were genotyped by pulsed-field gel electrophoresis. Susceptibility to vancomycin was assessed by the broth dilution method, population analysis, and time-kill studies and in the rabbit model of endocarditis. Strains SF1 and SF2 had similar genotypes, and the vancomycin MICs for the strains were ≤2 μg/ml. SF2 exhibited heterogeneous resistance to vancomycin. Vancomycin eradicated SF1 in the rabbit model of endocarditis, while SF2 persisted at pretreatment levels. Vancomycin treatment failure in this patient with endocarditis was attributable to heterogeneous resistance to vancomycin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a common cause of severe, hospital-acquired infections since the 1960s (1). Recently, however, strains of MRSA have moved to younger, healthier community populations (9, 11, 17). Because vancomycin is first-line therapy for serious MRSA infections, an increase in the incidence of both hospital-acquired and community-acquired infections may lead to a substantial increase in the rate of vancomycin usage.

Vancomycin treatment failures and slow clinical responses in cases of *S. aureus* endocarditis are well described (13, 16, 21) and preceded the first reports of failures of treatment for infections caused by *S. aureus* strains with intermediate susceptibility to vancomycin (vancomycin-intermediate *S. aureus* [VISA]; MICs, 8 to 16 μg/ml) (12, 15, 20; K. Hiramatsu, H. Hanaki, T. Ino, K. Yabuta, T. Oguri, and F. C. Tenover, Letter, J. Antimicrob. Chemother. **40**:135-136, 1997; M. C. Ploy, C. Grélaud, C. Martin, L. de Lumley, and F. Denis, Letter, Lancet **351**:1212, 1998). However, treatment failure is relatively common (the rate is perhaps as high as 10 to 20% in cases of endocarditis) (2), whereas VISA strains are quite rare. One hypothesis for the lack of a strong relationship between treatment failure and infection with a VISA strain is that methods commonly used for susceptibility testing are too insensitive to detect low-level (i.e., heterogeneous) vancomycin-intermediate *S. aureus* (hVISA). Strains of hVISA, therefore, not only may be progenitors of VISA but also may contribute to clinical failures in their own right (2).

We describe a patient with community-acquired MRSA endocarditis caused by a strain that was fully susceptible to van-comycin by a standard susceptibility testing methodology but who failed treatment with vancomycin. This failure was associated with the emergence of heterogeneous resistance to vancomycin in the patient, and the hVISA isolate failed to respond to vancomycin in a rabbit model of endocarditis.

CASE REPORT

A 47-year-old African-American male with a history of injection drug use presented to the San Francisco Veterans Affairs Medical Center in November 1998 with chief complaints of fever and weakness. Blood samples for culture were drawn on admission, and the cultures grew MRSA, hereafter referred to as strain SF1 (by the broth dilution method the oxacillin MIC was >16 μg/ml and the vancomycin MIC was 1 μg/ml); and a transesophageal echocardiogram showed a vegetation of 1 by 1 cm on the tricuspid valve. There were no other valvular vegetations. His course was complicated by septic pulmonary emboli and L2 to L4 vertebral osteomyelitis. He was treated with vancomycin at appropriate doses and with therapeutic levels for 6 weeks. In February 1999, the patient developed right elbow pain and swelling along with fever and renal insufficiency (serum creatinine level, 1.8 mg/dl). Two of two blood cultures and joint fluid all grew MRSA (strain SF2; by the broth dilution method the oxacillin MIC was >16 μg/ml and the vancomycin MIC was 2 μg/ml). The joint was surgically drained. A repeat transesophageal echocardiogram showed persistence of the tricuspid valve vegetation. Vancomycin (500 mg intravenously every 12 h, based on levels in serum) was restarted and rifampin (300 mg orally three times a day) was added to the regimen. A repeat echocardiogram showed no change in the previous tricuspid vegetation. He defervesced and antibiotics were continued for a second 6-week course of therapy. Follow-up blood cultures 1 week after the completion of antibiotics were negative, and the patient responded clini-

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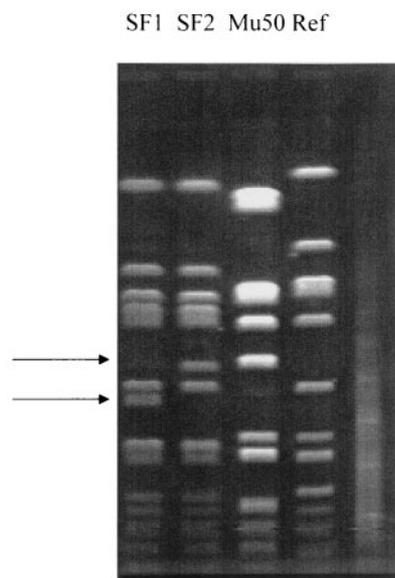
SF1 SF2 Mu50 Ref

FIG. 1. PFGE of SF1, SF2, Mu50, and a reference strain (Ref) with *Sma*I. Arrows indicate bands that distinguish SF1 and SF2 from each other.

cally. The patient was seen 3 months following the completion of therapy, and he had no evidence of recurrent endocarditis.

MATERIALS AND METHODS

Strain characteristics. Colonies of SF1 produced a surrounding zone of beta-hemolysis on blood agar, whereas SF2 was weakly beta-hemolytic. To further facilitate discrimination of SF1 from SF2 in growth competition experiments in the rabbit endocarditis model, rifampin-resistant mutants of SF1 and SF2 were obtained by a single passage of each parent strain on Trypticase soy agar (TSA) containing 1 μg of rifampin per ml. Two colonies from an inoculum of 1.6×10^7 CFU of strain SF1 and two colonies from an inoculum of 2.2×10^6 CFU of strain SF2 grew on the rifampin-containing agar after 24 h of incubation at 37°C. One rifampin-resistant clone was selected from each parent strain (clones SF1R and SF2R, respectively).

PFGE and in vitro susceptibility studies. Pulsed-field gel electrophoresis (PFGE) was performed as described previously (9) with the restriction endonuclease *Sma*I. Strains SF1 and SF2 were compared to strain Mu50 (generously provided by K. Hiramatsu, Juntendo University, Tokyo, Japan) and a nosocomial MRSA reference strain (from the University of Cologne, Cologne, Germany, from the collection of F. Perdreau-Remington) (9).

Vancomycin MICs were determined by standard microdilution method in cation-supplemented Mueller-Hinton broth at an inoculum of approximately 3×10^5 to 5×10^5 CFU/ml, according to the criteria of NCCLS (23). Other susceptibility studies were performed by the Kirby-Bauer disk diffusion method (18).

Population analysis was performed as described previously (14). Briefly, after SF1 and SF2 were grown overnight in Trypticase soy broth (TSB), serial 10-fold dilutions were plated onto Mueller-Hinton agar (MHA), TSA, or brain heart infusion agar (BHIA) containing 0, 1, 2, 3, 4, 5, 6, 7, or 8 μg of vancomycin per ml. The cultures were incubated for 48 h at 35°C. The threshold of detection of single colonies was 100 CFU.

Time-kill studies were performed in 10 ml of TSB at 37°C with and without vancomycin at concentrations of 6 and 10 μg/ml. One hundred-microliter samples of SF1 and SF2 were serially diluted 10-fold and cultured onto blood agar at 0, 4, and 24 h after inoculation of the broth culture. The colonies were counted after incubation for 24 h at 37°C.

Rabbit model of endocarditis. A rabbit model of aortic valve endocarditis in which a catheter is positioned across the valve was used (6). The experimental protocol used for the rabbit endocarditis studies was reviewed and approved by the University of California San Francisco Committee on Animal Research. The inoculum was standardized by growing each strain in TSB at 37°C overnight. Cells were harvested by centrifugation and resuspended in one-fourth of the original volume of phosphate-buffered saline plus 10% glycerol. The number of
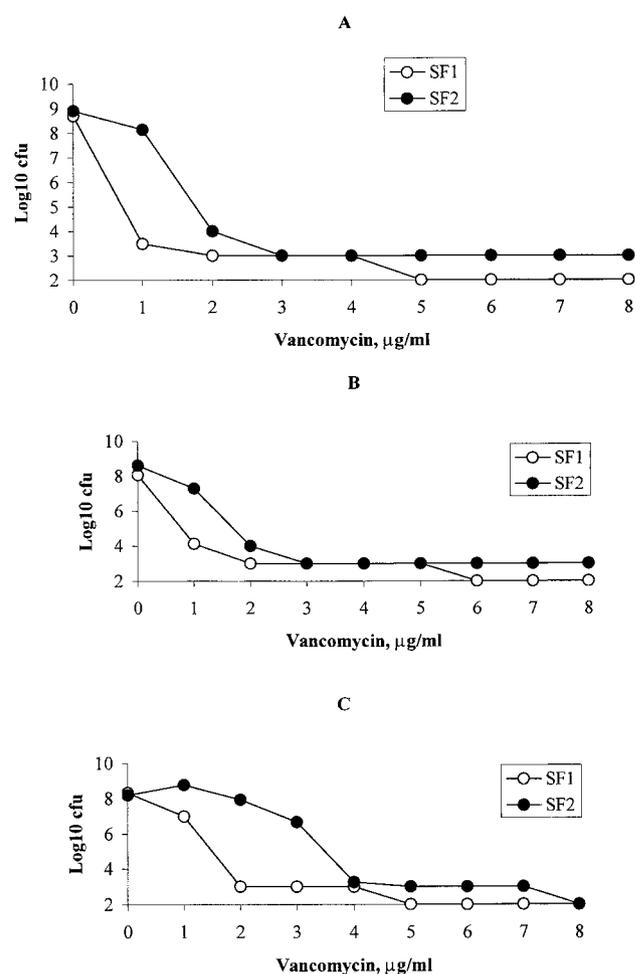
A

B

C

FIG. 2. Population analysis of SF1 and SF2 performed with vancomycin in MHA (A), TSA (B), or BHIA (C).

CFU per milliliter of suspension was determined by quantitative culture, and 0.5-ml aliquots were stored at −70°C. Forty-eight hours after catheter placement the rabbits were inoculated with 1 ml of a suspension of bacteria in 0.9% saline prepared by dilution of the frozen stock to achieve a final concentration of approximately 10^6 to 10^7 CFU/ml. The pretreatment burden of the organisms in the vegetations was determined by use of untreated control rabbits killed 18 h after inoculation. Rabbits treated with vancomycin received doses of 30 mg/kg of body weight intravenously every 12 h for 4 days. This is a typical dose that has been used in numerous prior studies (3–8) and that produces concentrations in serum of approximately 50 μg/ml 1 h after dosing and trough concentrations in serum of about 5 μg/ml. Vancomycin-treated rabbits were killed 18 h following administration of the last dose of vancomycin. Aortic valve vegetations were harvested, weighed, homogenized in 0.5 ml of 0.9% saline, and quantitatively cultured onto both blood agar (to examine the patterns of beta-hemolysis) and TSA plus 1 μg of rifampin per ml. The lower limit of detection of this method was approximately 10 CFU/g.

Statistical analysis. The number of bacteria in the vegetations was expressed as either the $\log_{10}$ CFU per gram or the $\log_{10}$ CFU per valve. Differences in bacterial counts were tested for statistical significance, defined as a *P* value of <0.05, by analysis of variance.

RESULTS

The pretreatment and relapse isolates (isolates SF1 and SF2, respectively) were closely related genotypically (22). The PFGE profiles demonstrated a two-band difference (indicated
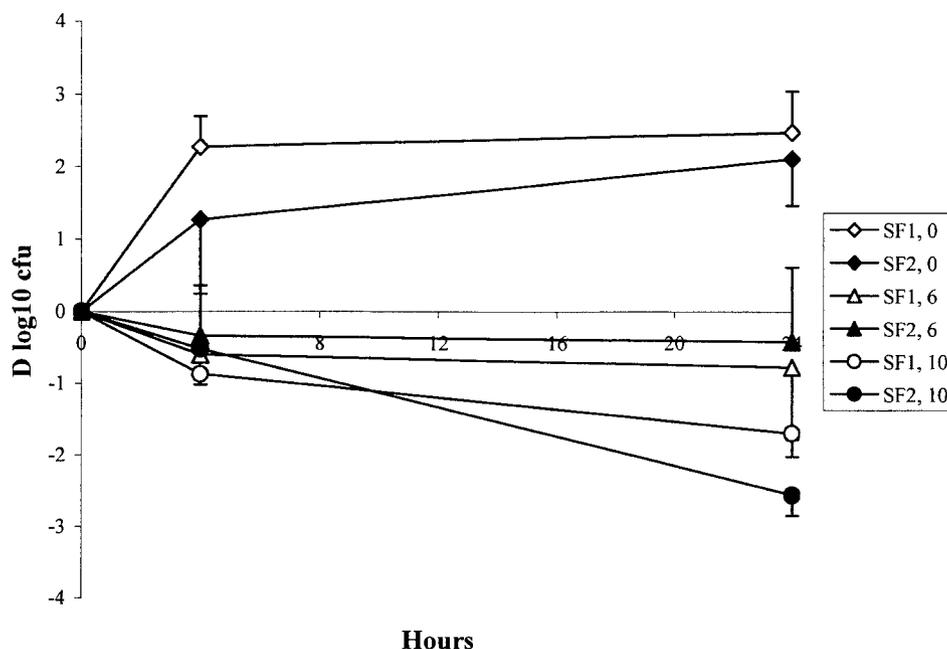
FIG. 3. Time-kill studies of SF1 and SF2 in TSB containing no vancomycin, 6 μg of vancomycin per ml, or 10 μg of vancomycin per ml. The values are the means of three experiments performed with inocula ranging between 10^4 and 10^7 CFU/ml. D $\log_{10}$ CFU, difference in the $\log_{10}$ CFU.

by the arrows in Fig. 1). Such a shift is the result of a single genetic event following the insertion of DNA with no change in the number of restriction sites. The profiles showed mobility patterns unrelated to VISA strain Mu50 and the reference MRSA strain, indicating that these strains are distantly related. SF1 and SF2 were both fully susceptible to vancomycin by standard broth dilution testing (MICs, 1 and 2 μg/ml, respectively). Although these values differed by only a single dilution, they were consistent and reproducible by repeat testing. Both strains were susceptible to clindamycin, erythromycin, tobramycin, and linezolid by the disk diffusion method.

By using TSA, BHIA, and MHA, the strain obtained after vancomycin treatment (strain SF2) survived in the presence of higher concentrations of vancomycin than the pretreatment strain (strain SF1). Differences were most pronounced with BHIA medium with vancomycin concentrations below 4 μg/ml. In the presence of vancomycin concentrations between 1 and 4 μg/ml, SF2 exhibited growth of 3 to 5 logs more than SF1. In the presence of vancomycin at concentrations above 4 μg/ml, SF2 survived at levels 1 log higher than SF1 (Fig. 2).

SF1 and SF2 exhibited similar susceptibilities to vancomycin in time-kill studies (Fig. 3). Vancomycin at 6 μg/ml resulted in less than a 1-$\log_{10}$ reduction in the number of CFU per milliliter at 24 h. Although 10 μg of vancomycin per ml was more effective, neither strain reached the 3-$\log_{10}$ cutoff for bactericidal activity.

Two series of experiments were conducted with the rabbit endocarditis model. In one series, rabbits were infected with a single strain, either SF1 or SF2, to determine the effect of vancomycin on the vegetation titers after 4 days of treatment. In the other series, rabbits were simultaneously coinfected with SF1 plus SF2R (a rifampin-resistant mutant of SF2 marked in order to readily distinguish it from SF1) or SF2 plus SF1R to

assess whether one isolate would outcompete the other in the vegetation during vancomycin treatment.

Pretreatment vegetation titers were very similar for rabbits infected with either strain (Table 1). After 4 days of vancomycin treatment, five of seven rabbits infected with strain SF1 had sterile vegetations and the mean titers were significantly reduced compared to those in all other groups ($P < 0.001$). Vancomycin-treated rabbits infected with strain SF2 had mean vegetation titers that were not statistically significantly different from the pretreatment titers. Similar results were observed for rabbits coinfected with two strains (Table 2). The pretreatment titers of SF1 and SF2R or SF1R and SF2 were virtually identical. Rabbits coinfected with SF1R and SF2 had a slight reduction in SF2 organism burden per valve compared to the pretreatment organism burden, but the difference did not achieve statistical significance. Only strain SF2 was detected in any of the vancomycin-treated rabbits ($P < 0.001$). Notably, rifampin-resistant mutant SF2R was also completely eradicated from the vegetations with vancomycin treatment, even though its susceptibility phenotype, including heterogeneous resistance to vancomycin, was not different from that of the

TABLE 1. Vegetation titers either before treatment or after 4 days of vancomycin therapy in aortic valves of rabbits infected with either SF1 or SF2

Strain	Titer ($\log_{10}$ CFU/g)	
	Pretreatment	Vancomycin
SF1	7.35 ± 1.15 (3)	0.21 ± 0.43 (7)
SF2	7.01 ± 1.2 (3)	6.9 ± 0.9 (5)

[a] The values are means ± standard deviations, with the numbers of rabbits tested given in parentheses.

TABLE 2. Number of organisms present in the inoculum mixture and on aortic valves of rabbits before treatment and after 4 days of vancomycin treatment

Inoculum mixture	Strain	Organism count[a]		
		Inoculum (log$_{10}$ CFU/g)	Pretreatment valves (log$_{10}$ CFU/valve)	Vancomycin-treated valves (log$_{10}$ CFU/valve)
SF1 + SF2R	SF1	5.69 ± 0.12 (4)	6.07 ± 0.51 (3)	0 ± 0 (5)
	SF2R	5.84 ± 0.18 (4)	5.64 ± 0.36 (3)	0 ± 0 (5)
SF1R + SF2	SF1R	5.76 ± 0.16 (4)	5.35 ± 0.78 (3)	0 ± 0 (5)
	SF2	5.80 ± 0.18 (4)	5.51 ± 0.28 (3)	4.52 ± 1.84 (5)

[a] The values are means ± standard deviations, with the numbers of rabbits tested given in parentheses.

parent strain. Additional population analysis failed to show any significant difference between SF2 and SF2R, suggesting that differences between the in vitro fitness of these strains, if any, were not detectable (data not shown).

DISCUSSION

We describe a patient with MRSA endocarditis who failed appropriate therapy with vancomycin. Several lines of evidence indicate that this treatment failure probably occurred because the patient was infected with a strain of hVISA that, under vancomycin selective pressure, emerged as a slightly more vancomycin-resistant variant that caused the infection to relapse. When studied in an animal model of endocarditis, the relapse strain (SF2) failed to respond to therapeutic doses of vancomycin that eradicated the initial strain (SF1) from aortic valve vegetations. That the two isolates are genotypically closely related is a strong indication that this is a true relapse and not a reinfection. The presence of a small resistant subpopulation could be demonstrated for the pretreatment isolate, isolate SF1. SF1 was apparently fully vancomycin susceptible (MIC, 1 μg/ml), and it was shown by population analysis that approximately 1 CFU in 10^5 to 10^6 SF1 organisms could grow in the presence of 4 to 8 μg of vancomycin per ml. The precipitous drop in the number of CFU growing in the presence of 1 to 2 μg of vancomycin per ml correlated well with the MIC of 1 μg/ml. In time-kill studies this strain was not killed in the presence of vancomycin at a concentration of 6 μg/ml, which over 24 h produced less than a 1-log$_{10}$ reduction in the starting inoculum. The relapse isolate, isolate SF2, was slightly less susceptible to vancomycin (MIC, 2 μg/ml); its behavior was similar to that of SF1 in time-kill studies.

The principal differences between the pretreatment and relapse isolates were detected by population analysis and in the endocarditis model. Depending on the medium used for the population analysis, in the presence of vancomycin at concentrations below 4 μg/ml, there was about a 4-log$_{10}$ difference in growth (numbers of CFU) between the pretreatment and relapse isolates. This difference was most apparent with BHIA compared to that detected with TSA or MHA. The relapse isolate also grew slightly better (1 order of magnitude difference) in the presence of vancomycin at concentrations above 4 μg/ml. In the endocarditis model, isolate SF2 was clearly less susceptible to vancomycin (which, after 4 days of vancomycin treatment, persisted at numbers near the pretreatment numbers) than the pretreatment isolate (which was largely eradicated). Vancomycin treatment failures in the rabbit model of endocarditis have been observed with documented VISA strains (19).

The dramatic difference in the response to vancomycin of SF1 compared to that of SF2 in the endocarditis model raises at least two questions. First, why was SF1 so readily eradicated from the rabbits, because it must have persisted in the patient in order for selection to occur? Second, why was the patient cured on the second attempt if the infecting strain was even more resistant to vancomycin? The answer to the first question may relate to the size and level of resistance of the resistant subpopulation at the site of infection. For SF1, the total number of organisms on the valve of a rabbit was on the order of 10^6; therefore, on the basis of population analysis, 1 to 10 CFU would be expected to survive in the presence of vancomycin at concentrations above 1 μg/ml. For SF2, the total number of organisms on the valve was the same, but 10^4 to 10^6 organisms would be expected to survive in the presence of vancomycin at concentrations between 1 and 4 μg/ml. With respect to the patient infected with SF1, given the size of the vegetation and the existence of a second site of infection, the overall organism burden stands to be substantially larger, increasing the chances for survival and further selection of the resistant subpopulation.

As to the second question, the use of rifampin in combination with vancomycin may account for the successful therapy the second time. The source of the relapse (the patient had both endocarditis and osteomyelitis) cannot be determined. It is tempting to speculate that it may have been the vertebral osteomyelitis and that the addition of rifampin was therapeutically decisive. Rifampin, in combination with vancomycin or fluoroquinolones, has been shown in animal models (10) to be superior to single agents. In a clinical trial of prosthesis-associated bone infection, patients who received rifampin in combination with a fluoroquinolone experienced fewer treatment failures than patients who did not receive rifampin (24). Recalling that the rifampin-resistant mutant of SF2 was eradicated by vancomycin in the endocarditis experiments may also indicate an interaction between vancomycin and rifampin resistance that adversely affects fitness.

These studies provide the first experimental data supporting the hypothesis that the failure of vancomycin to cure occasional cases of serious *S. aureus* infections, such as endocarditis, may be related to the presence of relatively vancomycin-resistant subpopulations of *S. aureus*, i.e., hVISA. Furthermore, our results suggest that population analysis performed with BHIA may be superior to other in vitro methods for

detection of this subclinical resistance and that it may be useful for prediction of the clinical response. Our report has one principal and very important limitation. The single case of vancomycin treatment failure reported on here may not be representative of other cases of vancomycin treatment failure. Nevertheless, we believe that this case adds weight to the hypothesis that treatment failures are related to the presence of vancomycin-resistant subpopulations in strains that otherwise appear fully vancomycin susceptible.

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