

## Gentamicin Improves the Activities of Daptomycin and Vancomycin against *Enterococcus faecalis* *In Vitro* and in an Experimental Foreign-Body Infection Model<sup>∇</sup>

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Received 3 February 2011/Returned for modification 14 April 2011/Accepted 22 July 2011

**For enterococcal implant-associated infections, the optimal treatment regimen has not been defined. We investigated the activity of daptomycin, vancomycin, and gentamicin (and their combinations) against *Enterococcus faecalis* *in vitro* and in a foreign-body infection model. Antimicrobial activity was investigated by time-kill and growth-related heat production studies (microcalorimetry) as well as with a guinea pig model using subcutaneously implanted cages. Infection was established by percutaneous injection of *E. faecalis* in the cage. Antibiotic treatment for 4 days was started 3 h after infection. Cages were removed 5 days after end of treatment to determine the cure rate. The MIC, the minimal bactericidal concentration (MBC) in the logarithmic phase, and the MBC in the stationary phase were 1.25, 5, and >20 µg/ml for daptomycin, 1, >64, and >64 µg/ml for vancomycin, and 16, 32, and 4 µg/ml for gentamicin, respectively. *In vitro*, gentamicin at subinhibitory concentrations improved the activity against *E. faecalis* when combined with daptomycin or vancomycin in the logarithmic and stationary phases. In the animal model, daptomycin cured 25%, vancomycin 17%, and gentamicin 50% of infected cages. In combination with gentamicin, the cure rate for daptomycin increased to 55% and that of vancomycin increased to 33%. In conclusion, daptomycin was more active than vancomycin against adherent *E. faecalis*, and its activity was further improved by the addition of gentamicin. Despite a short duration of infection (3 h), the cure rates did not exceed 55%, highlighting the difficulty of eradicating *E. faecalis* from implants already in the early stage of implant-associated infection.**

Implant-associated infections are caused by microorganisms growing attached on the device surface as biofilms (12, 31). The biofilm mode of growth represents a survival strategy through which microorganisms can attach and better resist antibiotics and the host defense system. In a biofilm, microorganisms can be up to 1,000-fold more resistant to antimicrobials than their planktonic counterparts (27). Therefore, infections in the presence of an implant are persistent and difficult to eradicate. Often, removal of all foreign-body material is performed, and it is replaced after several weeks, if needed. This approach causes considerable soft tissue damage and bone stock loss and requires repeated surgical interventions and a prolonged hospital stay (40). Therefore, new treatment options using antibiotics with increased activity against biofilms are being investigated, potentially allowing successful eradication of implant-associated infections without device removal.

Enterococci are increasingly recognized as the cause of health care-acquired infections, especially in immunocompromised hosts, such as critically ill patients and recipients of intravascular and extravascular implants (20, 25). Enterococci

cause 3 to 10% of periprosthetic joint infections, and the treatment is challenging due to high failure rates and common relapses (22, 40). Reasons for the common treatment failure of enterococcal implant-associated infections are not entirely clear. They may include the low killing rate of enterococci by β-lactam antibiotics and glycopeptides, antimicrobial tolerance, persistence in host cells (potentially mediated by the enterococcal surface proteins), and unavailability of potent antibiofilm agents, such as rifampin, which is active against staphylococcal biofilms (15, 26, 40). In addition, enterococci may acquire genes encoding resistance against β-lactams, glycopeptides, aminoglycosides, and oxazolidinones, which further complicates the treatment of enterococcal infections (17, 23, 38).

Daptomycin may be a treatment option for enterococcal biofilms due to its mechanism of action, particularly its rapid killing and concentration-dependent activity (9). We investigated the activity of daptomycin and vancomycin, alone and in combination with gentamicin, against planktonic and adherent *Enterococcus faecalis* *in vitro* and in a foreign-body infection model using guinea pigs. This model was previously used to evaluate treatment regimens of implant-associated infections caused by staphylococci and *Escherichia coli* (1, 5, 16, 30, 36, 39) but has not yet been employed for enterococcal infections.

(Parts of the results of the present study were presented at the 19th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Helsinki, Finland, 16 to 19 Sep-

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<sup>∇</sup> Published ahead of print on 1 August 2011.

tember 2009, and at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Boston, MA, 12 to 15 September 2010.)

## MATERIALS AND METHODS

**Study organism.** All experiments were performed with *E. faecalis* strain ATCC 19433. The strain has been shown to form biofilms (7). Bacteria were stored at  $-70^{\circ}\text{C}$  by using a cryovial bead preservation system (Microbank; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). To prepare the inoculum, one bead was spread on a blood agar plate and incubated at  $37^{\circ}\text{C}$ . One colony was resuspended in 5 ml of tryptic soy broth (TSB) and incubated at  $37^{\circ}\text{C}$ . Overnight cultures were adjusted to a turbidity of McFarland 0.5 (corresponding to  $\sim 5 \times 10^7$  CFU/ml). The exact inoculum was determined by CFU counting. For the infection inoculum, the overnight culture of *E. faecalis* was washed twice with sterile pyrogen-free 0.9% saline.

**Antimicrobial agents.** Daptomycin for injection was supplied by Novartis Pharma Schweiz AG (Bern, Switzerland). A stock solution of 50 mg/ml was prepared in sterile and pyrogen-free 0.9% saline. Vancomycin was delivered by Teva Pharma AG (Aesch, Switzerland) as 10 mg of powder in ampoules. The stock solution of 50 mg/ml was prepared in sterile and pyrogen-free 0.9% saline. Gentamicin was purchased from Essex Chemie AG (Lucerne, Switzerland) as a 40-mg/ml solution.

**Antimicrobial susceptibility.** The MICs and minimal bactericidal concentrations (MBCs) for daptomycin, vancomycin, and gentamicin were determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (10). A standard inoculum of  $\sim 3 \times 10^5$  CFU/ml was used. Serial 2-fold dilutions of the antimicrobials were prepared in cation-adjusted Mueller-Hinton broth (CAMHB) for the logarithmic growth phase. The MIC was defined as the lowest concentration of antibiotic that completely inhibited visible growth. The  $\text{MBC}_{10\%}$  was defined as the lowest antimicrobial concentration which killed  $\geq 99.9\%$  of the initial bacterial count (i.e.,  $\geq 3 \log_{10}$  CFU/ml) in 24 h (10). For stationary-growth-phase bacteria,  $\text{MBC}_{\text{stat}}$  was determined by using phosphate-buffered saline (PBS) supplemented with 0.1% TSB (34). Growth media for daptomycin studies were supplemented with 50 mg/liter  $\text{Ca}^{2+}$ . All experiments were performed in triplicate.

**Time-kill studies.** The activities of daptomycin, vancomycin, and gentamicin were investigated by time-kill studies under logarithmic- and stationary-growth-phase conditions. The initial inoculum was  $5 \times 10^5$  CFU/ml. Medium inoculated with bacteria only served as the growth control. CAMHB was used for the logarithmic growth phase, and PBS supplemented with 0.1% TSB was used for the stationary growth phase. Growth media for daptomycin studies were supplemented with 50 mg/liter  $\text{Ca}^{2+}$ . Tubes were incubated under static conditions at  $37^{\circ}\text{C}$ , and aliquots were taken after 0, 2, 4, 6, 8, and 24 h. To avoid antibiotic carry-over, samples were washed with sterile 0.9% saline and appropriate dilutions were spread on Muller-Hinton agar. The experiments were performed in triplicate, and means and standard deviations were plotted in a semilogarithmic graph. A bactericidal effect was defined as a  $\geq 3\text{-log}_{10}$  ( $\geq 99.9\%$ ) reduction of the initial bacterial count in 24 h.

**Microcalorimetry.** To test the growth inhibition by antimicrobials and their combinations, measurements of bacterial heat production were performed with a 48-channel batch calorimeter (thermal activity monitor, model 3102 TAM III; TA Instruments, New Castle, DE). Microcalorimetry is a highly sensitive and accurate method for measurement of heat production generated by microbial growth. The effect of antibiotics on bacterial growth can be evaluated by the delay of heat flow and reduction of the heat flow peak compared to results with the growth control without the presence of antibiotics (2, 3, 6, 28, 29, 33). Calorimeter ampoules with 3 ml of CAMHB supplemented with  $\text{Ca}^{2+}$  containing daptomycin, vancomycin, or gentamicin at  $0.125 \times \text{MIC}$ ,  $0.25 \times \text{MIC}$ ,  $0.5 \times \text{MIC}$ , and  $1 \times \text{MIC}$  were inoculated with  $3 \times 10^5$  CFU/ml. In combination studies, daptomycin and vancomycin at  $0.25 \times \text{MIC}$  were combined with gentamicin in concentrations of  $0.125 \times \text{MIC}$ ,  $0.25 \times \text{MIC}$ , and  $0.5 \times \text{MIC}$ . The heat flow at  $37^{\circ}\text{C}$  was measured for 24 h. The results were plotted as heat flow over time.

**Animal model.** Male albino guinea pigs (Charles River, Sulzfeld, Germany) were kept in the Animal Facility of the University Hospital Lausanne, Lausanne, Switzerland, and the experiments were performed according to the regulations of Swiss veterinary law. Guinea pigs were weighed daily to monitor their well-being during the experiment. Short-term studies (4-day treatment) were performed in a foreign-body cage model for guinea pigs developed by Zimmerli et al. (41). Four sterile polytetrafluoroethylene (Teflon) cages with 130 regularly spaced perforations 1 mm in diameter (Angst-Pfister AG, Zürich, Switzerland) were

subcutaneously implanted in flanks of the guinea pigs (450 to 550 g) under aseptic conditions. After complete wound healing (approximately 2 weeks after implantation), the sterility of the cages was confirmed by culturing aspirated cage fluid on blood agar plates. The bacterial inoculation was performed only in initially sterile cages.

**Pharmacokinetic studies.** Pharmacokinetic studies for daptomycin and vancomycin were performed in previous studies (5, 16). For gentamicin pharmacokinetic studies, cage fluid was aspirated from noninfected animals over 24 h (2, 4, 6, 10, and 24 h) following intraperitoneal administration of a single dose of gentamicin at 10 mg/kg. Three guinea pigs were used; i.e., 12 cages. At each time point, 150- $\mu\text{l}$  aliquots of cage fluid were aspirated from two cages from each animal (i.e., six replicates per time point). The collected fluid was centrifuged ( $2,100 \times g$  for 7 min) at  $4^{\circ}\text{C}$  and the supernatant was stored at  $-20^{\circ}\text{C}$  until further analysis. The means  $\pm$  standard deviations (SD) of the peak concentration ( $C_{\text{max}}$ ), the time to reach  $C_{\text{max}}$  ( $T_{\text{max}}$ ), and the trough concentration at 24 h after dosing ( $C_{\text{min}}$ ) were determined.

**Determination of drug concentrations.** Gentamicin concentrations in cage fluid were determined by an agar plate diffusion bioassay with *Bacillus subtilis* strain ATCC 6051 as the indicator organism. Mueller-Hinton agar (Difco, BD, Le Pont de Claix, France) was suspended in sterile pyrogen-free water, and the mixture was autoclaved. The medium was then cooled to  $45^{\circ}\text{C}$ , inoculated with the overnight culture of the indicator organism (100  $\mu\text{l}$  per 165 ml of medium), and poured into large assay plates (30 cm by 30 cm). Calibration curves were plotted for the assay plate, and the regression-fitting equation was extrapolated. The standard solutions were prepared in 0.9% NaCl by preparing 2-fold serial dilutions. One hundred microliters of the cage fluid samples and duplicates of the gentamicin standard solutions were spotted into holes punched in the assay plates, and the plates were incubated overnight at room temperature, followed by incubation at  $37^{\circ}\text{C}$  for 24 h. The diameter of the inhibition zone was measured with calipers. The bioassay detection limit corresponded to the gentamicin  $0.5 \times \text{MIC}$  of the indicator organism (i.e.,  $0.5 \mu\text{g/ml}$ ).

**Antimicrobial treatment.** Cages were infected by percutaneous inoculation of 200  $\mu\text{l}$  of *E. faecalis* containing  $3 \times 10^4$  CFU. The exact infection inoculum was determined by colony counts. Three hours after infection, quantitative cultures of aspirated cage fluid were performed, followed by the start of antimicrobial treatment. In each of the following five treatment regimens, three animals were randomized, each having 4 cages (i.e., 12 cages per treatment regimen): control group (no antibiotic treatment); daptomycin, 40 mg/kg of body weight; daptomycin, 40 mg/kg plus gentamicin 10 mg/kg; vancomycin, 15 mg/kg; vancomycin, 15 mg/kg plus gentamicin 10 mg/kg; and gentamicin, 10 mg/kg. All antimicrobial agents were given intraperitoneally twice daily, except daptomycin, which was given once daily. The duration of treatment was 4 days.

**Antimicrobial efficacy on planktonic and adherent *E. faecalis*.** Cage fluid was aspirated before the start of treatment, during treatment (immediately before the application of the last dose), and 5 days after completion of treatment. The bacterial counts were expressed as  $\log_{10}$  CFU/ml of cage fluid. The quantification limit for bacteria in the cage fluid was set to 200 CFU/ml ( $>10$  CFU in 50  $\mu\text{l}$  of a 10-fold dilution). The clearance rate (as a percentage) was defined as the number of cage fluid cultures without growth of *E. faecalis* divided by the total number of cage fluid cultures in the individual treatment group. To determine the antimicrobial efficacy against adherent bacteria, animals were sacrificed 5 days after completion of treatment, and cages were explanted under aseptic conditions and incubated in 5 ml TSB. After a 48-h incubation of the cages in TSB, aliquots of 100  $\mu\text{l}$  were spread on a blood agar plate and incubated at  $37^{\circ}\text{C}$  for additional 48 h. The treatment efficacy against adherent bacteria was expressed as the cure rate (as a percentage), defined as the number of cages without *E. faecalis* growth divided by the total number of cages in the individual treatment group.

**Statistical calculations.** Comparisons for continuous variables were performed by the Mann-Whitney U test. For all tests, differences were considered significant when  $P$  values were  $<0.05$ . The graphs in the figures were plotted with Prism (version 5.0a) software (GraphPad Software, La Jolla, CA).

## RESULTS

***In vitro* antimicrobial susceptibility.** Table 1 summarizes the *in vitro* susceptibility of *E. faecalis*. Daptomycin and vancomycin had low MIC values (1.25 and 1  $\mu\text{g/ml}$ , respectively) but did not kill the bacteria in the stationary growth phase. Daptomycin showed bactericidal activity in the logarithmic phase ( $\text{MBC}_{10\%}$ , 5  $\mu\text{g/ml}$ ), and gentamicin was killing *E. faecalis* in

TABLE 1. *In vitro* susceptibility of *E. faecalis* strain ATCC 19433 against daptomycin, vancomycin, and gentamicin as determined by broth microdilution<sup>a</sup>

Antimicrobial	MIC (μg/ml)	MBC <sub>log</sub> (μg/ml)	MBC <sub>stat</sub> (μg/ml)
Daptomycin	1.25	5	>20
Vancomycin	1	>64	>64
Gentamicin	16	32	4

<sup>a</sup> MBC<sub>log</sub>, minimal bactericidal concentration in the logarithmic growth phase; MBC<sub>stat</sub>, minimal bactericidal concentration in the stationary growth phase. The results are mean values from triplicate measurements.

both the logarithmic (MBC<sub>log</sub>, 32 μg/ml) and the stationary (MBC<sub>stat</sub>, 4 μg/ml) growth phases.

**Time-kill studies.** Figure 1 demonstrates killing of bacteria in the logarithmic growth phase. Daptomycin and gentamicin (Fig. 1A and C) show a dose-dependent activity at concentrations from 0.25× to 16× MIC, both being bactericidal (i.e., 3-log reduction) at 4× MIC. Vancomycin (Fig. 1B), on the other hand, showed no bactericidal effect at concentrations of up to 16× MIC. Figure 2 shows the antimicrobial activity against bacteria in the stationary phase. Daptomycin showed no bactericidal effect at up to 16× MIC (Fig. 2A), whereas vancomycin reduced bacterial counts by approximately 3-log at 1× to 16× MIC (Fig. 2B). Gentamicin was the most active antimicrobial agent, being bactericidal at 0.5× MIC (Fig. 2C). When combining daptomycin at 0.25× MIC with gentamicin at different subinhibitory concentrations, the antimicrobial activity was increased against *E. faecalis* in the logarithmic phase (Fig. 3A). This additive effect was less pronounced when combining vancomycin and gentamicin (Fig. 3B). In the stationary phase, gentamicin improved the killing of *E. faecalis* by daptomycin and vancomycin (Fig. 3C and D), with a time-kill curve resembling that of gentamicin alone.

***In vitro* activity on *E. faecalis* evaluated by microcalorimetry.**

The effect of daptomycin, vancomycin, and gentamicin at subinhibitory concentrations and 1× MIC on *E. faecalis* growth-related heat production was investigated using a batch calorimeter. The drugs were tested alone (Fig. 4A to C) and in combination with gentamicin (Fig. 4D and E) at subinhibitory concentrations. Since bacteria in the stationary phase do not produce detectable heat, the experiment was performed only with logarithmic phase bacteria. A fixed concentration of daptomycin and vancomycin at 0.25× MIC was combined with gentamicin at 0.125×, 0.25×, and 0.5× MIC.

The growth control had a maximal heat flow peak of 315 μW at 5.6 h of incubation. The activity of the used antibiotics resulted in decreased and delayed peaks of the heat flow curves. At 1× MIC, heat production was completely inhibited for 24 h by all antibiotics. Daptomycin alone was more active in the suppression of growth-related heat production of *E. faecalis* than vancomycin and gentamicin alone using the same concentrations. Whereas a delay in the heat flow peak was observed for daptomycin already at 0.125× MIC (Fig. 4A), the inhibitory effect of vancomycin (Fig. 4B) and gentamicin (Fig. 4C) was present only at 0.5× MIC.

The addition of gentamicin at a low concentration (0.125× MIC) enhanced the antienterococcal activity of both daptomycin and vancomycin (Fig. 4 D and E), whereas gentamicin alone showed a minimal antimicrobial effect at this concentra-

tion (Fig. 4C). The combination of daptomycin and gentamicin was more active in the suppression of growth-related heat production than the combination of vancomycin and gentamicin using the same fractional concentrations of the MIC.

**Pharmacokinetics of gentamicin in the cage fluid.** After an intraperitoneal application of gentamicin (10 mg/kg), a C<sub>max</sub> (mean ± SD) of 6.2 ± 1.6 μg/ml was reached after 2 h (T<sub>max</sub>).

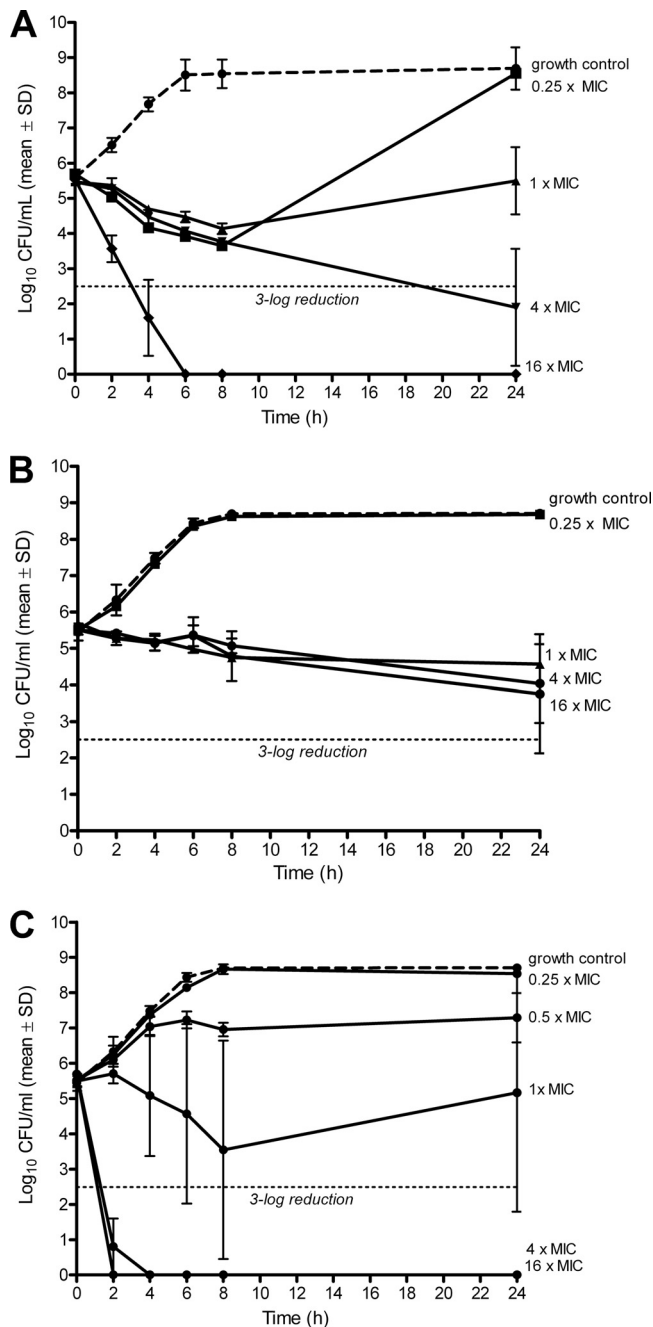


FIG. 1. Time-kill studies for daptomycin (A), vancomycin (B), and gentamicin (C) in logarithmic growth phase. Values represent means and standard deviations (SD) of triplicate measurements. The limit of detection was 200 CFU/ml. The horizontal dashed line represents the reduction of 3 log<sub>10</sub> CFU/ml of the initial inoculum. The dashed line represents the growth control.



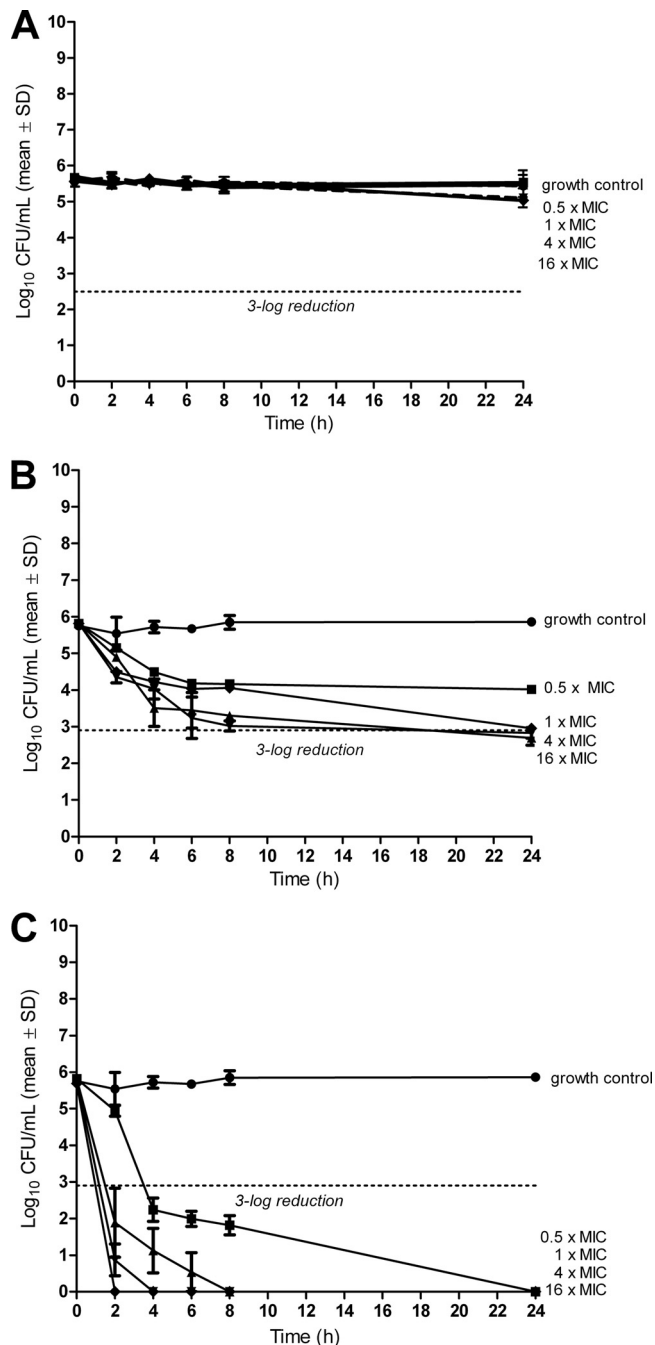


FIG. 2. Time-kill studies for daptomycin (A), vancomycin (B), and gentamicin (C) in stationary growth phase. Values represent means and standard deviations (SD) of triplicate measurements. The limit of detection was 200 CFU/ml. The horizontal dashed line represents the reduction of  $3 \log_{10}$  CFU/ml of the initial inoculum.

The concentration of gentamicin in the cage fluid 24 h after application ( $C_{\min}$ ) was 0  $\mu\text{g/ml}$ .

**In vivo activity on planktonic *E. faecalis*.** Figure 5 shows the bacterial load in the cage fluid throughout the experiment. After 3 h of infection, before the start of treatment, the bacterial load in the aspirated cage fluid was  $3.5 \log_{10}$ . No spontaneous clearance of planktonic bacteria from the cage fluid in

the untreated group was observed, and the bacterial load remained unchanged during the experiment ( $\sim 3.5 \log_{10}$ ). Five days after completion of therapy, the planktonic bacteria in the cage fluid had decreased by  $1.9 \log_{10}$  with daptomycin, by  $2.5 \log_{10}$  with vancomycin, and by  $2.6 \log_{10}$  with gentamicin. The combination of gentamicin and daptomycin was the most efficient regimen in killing planktonic *E. faecalis*, with complete clearance of the cage fluid (Fig. 6A). When a combination of gentamicin with vancomycin was used, planktonic bacteria decreased by  $1.8 \log_{10}$  and clearance of planktonic bacteria was achieved in 50% of cage fluid samples.

**In vivo activity on adherent *E. faecalis*.** Figure 6B shows the cure rates of different antibiotic regimens against adherent bacteria. Without antibiotics (control animals), the cure rate was 0%. Daptomycin, vancomycin, and gentamicin as monotherapy eradicated the adherent bacteria in 25%, 17%, and 50% of cages, respectively. In combination with gentamicin, the cure rate increased to 55% (daptomycin) and 33% (vancomycin).

## DISCUSSION

The management of implant-associated infections is challenging, and clinical experience is limited, especially with retention of the infected device (40). Therefore, we compared the *in vitro* and *in vivo* activities of daptomycin and vancomycin, alone and in combination with gentamicin, against planktonic and adherent *E. faecalis*. The chosen *E. faecalis* strain is representative of the current epidemiological situation, with susceptibility to lipopeptides and glycopeptides, without demonstrating high-level resistance to gentamicin (MIC, 16  $\mu\text{g/ml}$ ). However, the prevalence of multiresistant enterococcal strains may increase in the future. Therefore, daptomycin was chosen as one of the treatment regimens. Daptomycin is a promising agent due to its mechanism of action, particularly its rapid killing and concentration-dependent activity (9). According to CLSI (11) and EUCAST (<http://www.eucast.org/>) guidelines, the majority of current clinical isolates of enterococci are susceptible to daptomycin (MIC,  $\leq 4 \mu\text{g/ml}$ ). Moreover, daptomycin is not associated with cross-resistance to other antimicrobials and is also active against most resistant isolates, including vancomycin-resistant enterococci (VRE) (9). However, the risk of emergence of daptomycin resistance during treatment of enterococcal infections is of concern. The genetic basis for daptomycin resistance in enterococci was recently reviewed (21).

The *in vitro* experiments using time-kill studies and measurement of growth-related bacterial heat production (microcalorimetry) showed an improved antibacterial activity of daptomycin and vancomycin when in combination with gentamicin. This effect was observed in the logarithmic phase, whereas in the stationary phase, the killing effect of gentamicin combination represented mostly the pronounced killing effect of gentamicin alone. Based on these *in vitro* results, gentamicin was included in the treatment combination regimens in the experimental studies in the guinea pig model. The animal model was adapted from the previously described setting (1, 16, 30, 39) by reducing the infection inoculum to  $3 \times 10^4$  CFU/cage and shortening the duration of infection to 3 h. Despite the fact that we have not demonstrated the formation of biofilm on explanted cages, we assume that adherent bacteria in biofilm

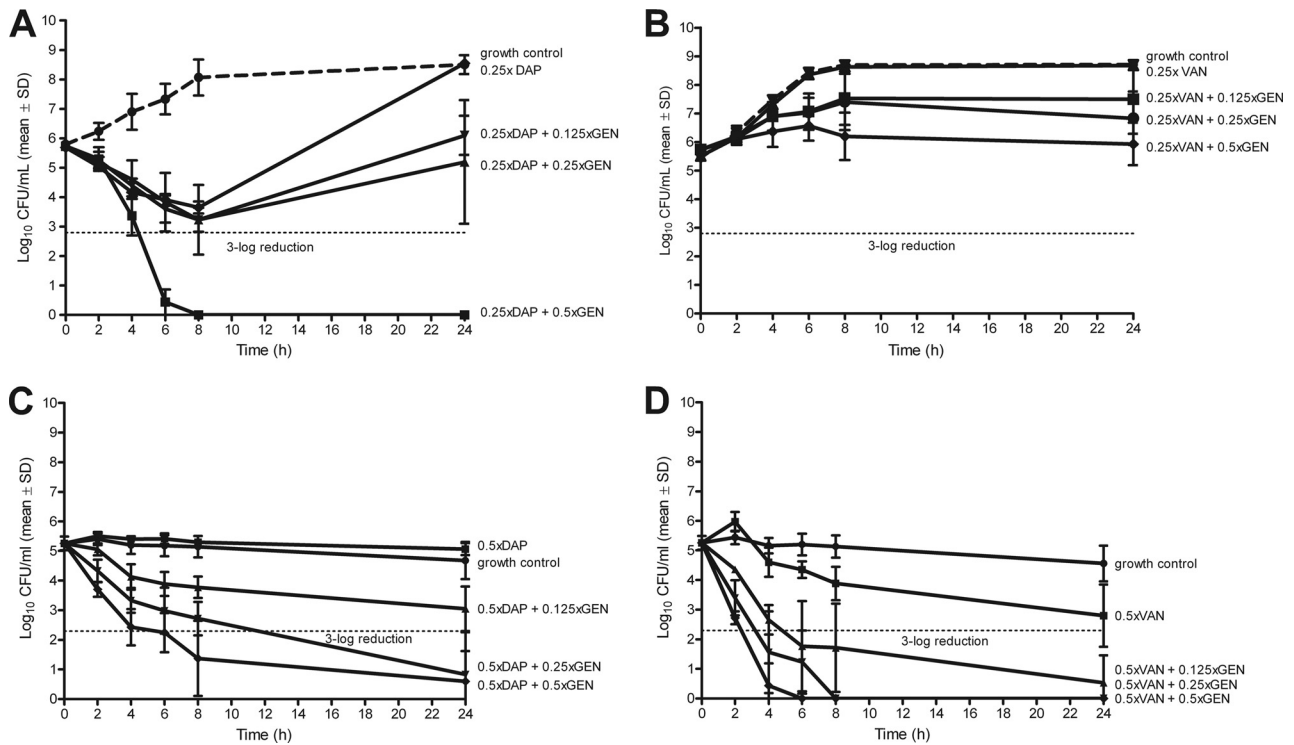


FIG. 3. Time-kill curves of *E. faecalis* in logarithmic (A and B) and stationary (C and D) growth phase with daptomycin (DAP; A and C) and vancomycin (VAN; B and D) combined with gentamicin (GEN) at different concentrations. Values represent means and standard deviations (SD) of triplicate measurements. The horizontal dashed line represents the reduction of 3 log<sub>10</sub> CFU/ml of the initial inoculum.

are responsible for frequent treatment failure. With the 24-h duration of infection, no cure of infected cages was observed (data not shown). Under adapted experimental conditions (using a short infection duration), cure rates did not exceed 55%.

This highlights the propensity of *E. faecalis* to rapidly cause persistent infections, which are difficult to eradicate despite a low MIC of all tested antimicrobial drugs. In staphylococcal infection, cure rates were significantly improved by combina-

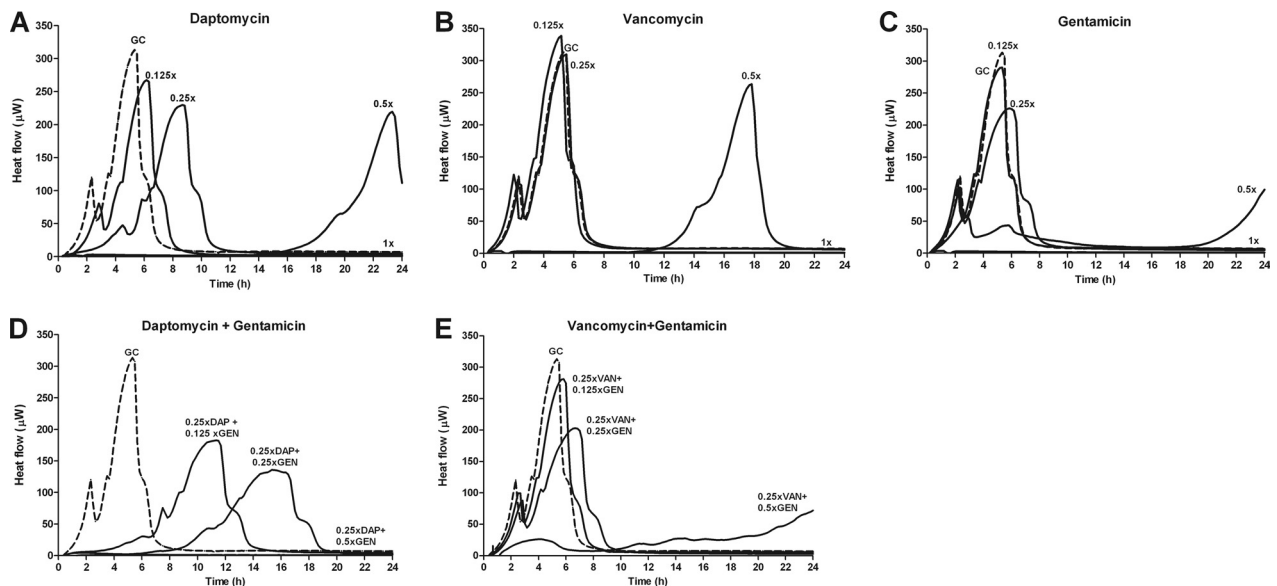


FIG. 4. Calorimetric measurements of heat produced by *E. faecalis* during 24 h exposed to different concentration of daptomycin (DAP; A), vancomycin (VAN; B), gentamicin (GEN; C) and their combinations (D and E). All drugs were measured at concentrations of 0.125× MIC, 0.25× MIC, 0.5× MIC and 1× MIC. The combinations were tested with fixed concentrations of daptomycin and vancomycin (0.25× MIC) with three concentrations of gentamicin (0.125×, 0.25×, and 0.5× MIC). GC, growth control (dashed line).

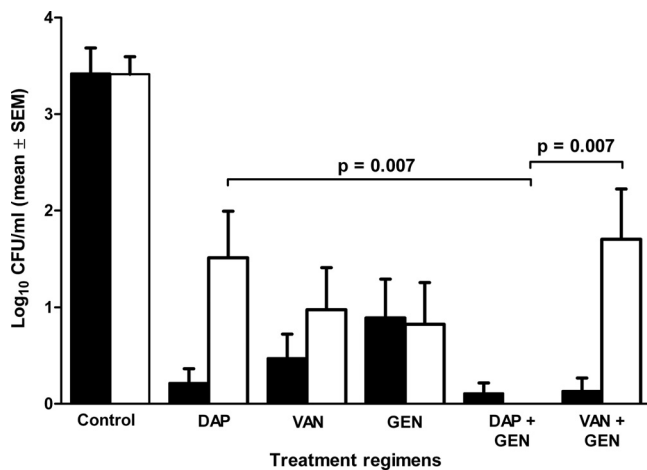


FIG. 5. Bacterial load of planktonic bacteria in cage fluid during treatment (black bars) and 5 days after treatment (white bars). Values are means  $\pm$  standard errors of the means (SEM). DAP, daptomycin; VAN, vancomycin; GEN, gentamicin.

tion with rifampin, which acts against biofilms (1, 16, 31, 32, 35, 42). In addition, combination regimens may prevent the emergence of resistant strains.

In our animal studies, the injected dose of daptomycin in guinea pigs (40 mg/kg) corresponds to a dose of 8 mg/kg in humans (4, 13, 37), which is higher than that currently recommended for staphylococcal infection in clinical practice (19). The most efficient regimen for killing planktonic *E. faecalis* was the combination of daptomycin and gentamicin, followed by gentamicin alone, as observed in the *in vitro* experiments. When evaluating the activity against adherent bacteria, daptomycin with gentamicin showed the highest cure rate (55%), whereas vancomycin with gentamicin showed a lower cure rate (33%). Gentamicin alone was able to eradicate the biofilm in 50% of the cages. However, gentamicin monotherapy is not recommended in clinical use because of the risk of emergence of resistance. This was shown in a study by Lefort et al. (18), in which gentamicin alone showed a significant reduction of the number of *E. faecalis* bacteria in the vegetation in a rabbit model of endocarditis but selected gentamicin-resistant mutants.

The activity of gentamicin combination against enterococcal biofilms was investigated in several laboratory and clinical studies. *In vitro* enterococcal biofilms were inhibited by high concentrations of ampicillin, vancomycin, and linezolid. In combination with gentamicin, these agents significantly reduced the MIC, MBC, and minimal biofilm inhibition concentration (MBIC) against several tested enterococcal isolates (24). Furthermore, in a rabbit endocarditis model, improved activity of daptomycin was observed against *Enterococcus faecalis* when combined with gentamicin (8). However, in a retrospective clinical study, including enterococcal prosthetic joint infections, no benefit for the treatment outcome of infections of the addition of gentamicin to aminopenicillins or glycopeptides was observed (14). Whether daptomycin in combination with gentamicin is superior to other antimicrobial regimens against adherent enterococci, including vancomycin-resistant enterococci (VRE), and whether the infected im-

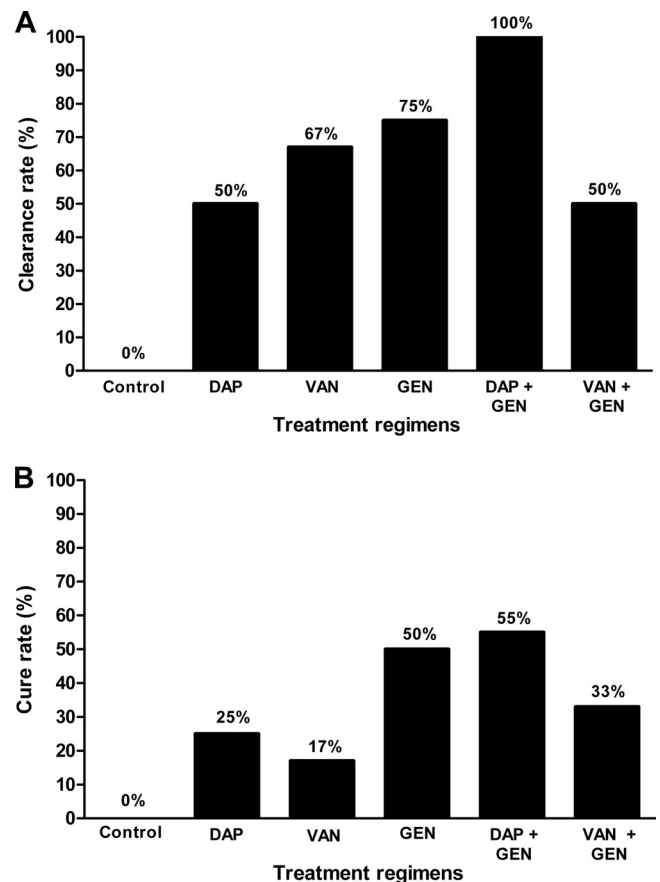


FIG. 6. Clearance rate of planktonic bacteria in cage fluid (A) and cure rate of adherent bacteria from explanted cages (B). DAP, daptomycin; VAN, vancomycin; GEN, gentamicin. The numbers above the columns indicate the clearance and cure rate, respectively.

plants can be retained with this combination, needs to be investigated in future clinical studies. The optimal regimen against strains with a high level of resistance to gentamicin remains to be determined.

#### ACKNOWLEDGMENTS

This study was supported by the Swiss National Science Foundation (K-32K1\_120531) and by an educational grant from Novartis Pharma AG, Basel, Switzerland.

We thank Alain Bizzini for critical reviews of the manuscript, Anne-Katrin John for practical advice, and Zarko Rajacic for laboratory assistance.

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