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Antimicrobial Susceptibility and Molecular Epidemiology of β-Lactamase-Producing, Aminoglycoside-Resistant Isolates of *Enterococcus faecalis*

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β-Lactamase-producing (BL^+), aminoglycoside-resistant (AR) *Enterococcus faecalis* is endemic in our hospital, having caused widespread colonization and infection. Suitable therapy for infections caused by these organisms has been problematic. We compared the antimicrobial and bactericidal activities, by broth macrodilution and time-kill methods, of several antibiotics, alone and in combination, against BL^+, AR isolates of *E. faecalis* and determined the transmissibility of antibiotic resistance markers. Ampicillin-sulbactam, imipenem, daptomycin, and ciprofloxacin were the most active antibiotics with MICs for 90% of isolates tested of 2, 1, 2, and 1 μg/ml, respectively, against inocula of 10^3 and 10^5 CFU/ml. Little inoculum effect was noted with imipenem, vancomycin, daptomycin, or ciprofloxacin, while the addition of sulbactam to ampicillin partially inhibited the effect of the increased inoculum. Penicillin-sulbactam and ampicillin-sulbactam combinations in a 2:1 ratio were most frequently bactericidal (≥3-$\log_{10}$-unit decrease in bacterial titers at 24 h for 13 of 20 isolates), followed by daptomycin (8 of 20 isolates) and ciprofloxacin (2 of 20 isolates). Bactericidal activity was not demonstrated for imipenem or teicoplanin. β-Lactamase production and aminoglycoside resistance were associated with a 60- to 65-MDa plasmid which was easily transferred to a plasmid-free *E. faecalis* recipient. The 840-bp β-lactamase gene probe hybridized to purified plasmid DNA from BL^+ donor isolates of *E. faecalis* and transconjugants but not from BL^- isolates. Ampicillin-sulbactam and daptomycin (an investigational antibiotic) seem to be reasonable choices for the empiric therapy of presumed enterococcal infections in hospitals in which BL^+, AR *E. faecalis* strains are isolated. Their use should ideally be supported by tests for bactericidal activity.

Despite the widespread use of penicillin for many decades, the first β-lactamase-producing (BL^+) strain of *Enterococcus faecalis* was not isolated until 1983 (10). Since then, additional isolates have been reported sporadically from widely separated geographic areas (4, 9, 13–15). Most BL^+ isolates have demonstrated high-level aminoglycoside resistance because of the presence of aminoglycoside-inactivating enzymes (4, 9, 13–15). The production of both β-lactamase and aminoglycoside-inactivating enzymes is mediated by plasmid-borne genes and can be cotransferred to suitable recipients (9, 10, 13). While the emergence of BL^+, aminoglycoside-resistant (AR) *E. faecalis* isolates is an ominous evolutionary event and would seem to abrogate standard forms of therapy, i.e., combinations of β-lactam and aminoglycoside antibiotics, few clinical infections have been reported (12, 16).

BL^+, AR *E. faecalis* isolates are endemic in our hospital, accounting for approximately 11% of all enterococci recently isolated in the microbiology laboratory. Both colonization and severe clinical infections have been observed (21). The isolation of large numbers of such isolates at a single institution has afforded us the opportunity to evaluate potential alternative forms of antibiotic therapy. We performed susceptibility testing by broth dilution and time-kill methods and compared the activities of several antibiotics, alone and in combination, against the BL^+, AR *E. faecalis* isolates. We also characterized the genetic basis for β-lactamase production and transmissibility.

(This study was presented in part at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [7].)

MATERIALS AND METHODS

Bacterial strains and β-lactamase activity. All catalase-negative, gram-positive cocci growing on bile-esculin agar and in 6.5% NaCl broth were screened as enterococci by the microbiology laboratory of our hospital. Isolates were identified as *E. faecalis* by using the API Rapid Strep system (Analytab Products, Plainview, N.Y.) and tested for β-lactamase production with nitrocefin disks (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). High-level resistance to aminoglycoside was detected by growth on agar plates containing gentamicin, 500 μg/ml, and streptomycin, 2,000 μg/ml. Sixty BL^+, AR *E. faecalis* isolates were found among 534 isolates recovered over a 10-month period.

Antibiotics. The antibiotics evaluated in this study (and the sources) included the following: penicillin G, ampicillin, and streptomycin (Sigma Chemical Co., St. Louis, Mo.); ampicillin-sulbactam and sulbactam (Pfizer, Inc., Groton, Conn.); imipenem (Merck Sharp & Dohme, West Point, Pa.); gentamicin (Schering Corp., Bloomfield, N.J.); vancomycin and daptomycin (Eli Lilly & Co., Indianapolis, Ind.); teicoplanin and rifampin (Merrell Dow, Cincinnati, Ohio); and ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.).

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Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed by broth macrodilution in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) supplemented with calcium, 50 mg/liter, and magnesium, 25 mg/liter. Inocula of 10^3, 10^5, and 10^7 CFU/ml, which were prepared from overnight broth cultures, were evaluated. The lowest concentration of antibiotic that prevented visible growth after 18 h of incubation at 37°C was defined as the MIC. The MBC was determined by transferring 0.01 ml from each clear tube to the surface of an antibiotic-free blood agar plate and incubating for 18 h at 37°C. The concentration of antibiotic resulting in a ≥99.9% reduction in the inoculum was defined as the MBC. Strain *E. faecalis* ATCC 29212 was tested in parallel; culture tubes without antibiotic served as growth controls. Ten β-lactamase-negative (BL^-), aminoglycoside-susceptible isolates of *E. faecalis* were also tested.

Time-kill studies. Twenty randomly selected BL^+, AR isolates of *E. faecalis* were selected for study. Time-kill experiments were performed in flasks containing MHB, with and without antibiotics. Antibiotic concentrations were chosen to equal the MIC or 4× the MIC for each organism and, in every instance, could be exceeded by the concentrations achievable in serum. An inoculum of approximately 5×10^6 CFU/ml prepared from an overnight (18-h) broth culture was added to each flask containing MHB and incubated at 37°C. Samples were removed at 0, 6, 24, and 48 h, serially diluted, and spread in 0.1-ml aliquots on the surface of antibiotic-free blood agar plates. Time-kill curves were constructed for each isolate by determining the viable bacterial counts at each interval. Bactericidal activity was defined as a ≥3-$\log_{10}$-unit reduction in CFU per milliliter at 24 h when compared with that in the original inoculum. Antibiotic carryover was evaluated as follows. Samples of MHB containing known concentrations of bacteria (10^2 to 10^5 CFU/ml) grown with and without antibiotics were inoculated onto antibiotic-free Mueller-Hinton agar plates. The difference in colony counts of organisms grown with and without antibiotics was ≤5% on multiple determinations, thereby eliminating the possibility of antibiotic carryover.

Conjugation studies. The conjugal transfer of antibiotic resistance determinants was evaluated by a filter paper mating technique (2) by using 20 randomly selected BL^+, AR isolates of *E. faecalis* as donors and *E. faecalis* JH2-2 as the recipient (5). Broth cultures of donor and recipient cells were grown to the stationary phase, mixed in a ratio of 100 μl of donor cells to 900 μl of recipient cells, collected in a 0.45-μm-pore-size nitrocellulose filter, and incubated upright on nonselective Mueller-Hinton agar for either 4 or 18 h at 37°C. Cells were then resuspended and spread on an agar plate containing gentamicin, 500 μg/ml; fusidic acid, 20 μg/ml; and rifampin, 100 μg/ml. Two transconjugants from each of the 20 mating experiments were subcultured twice on selective medium and tested for β-lactamase production. The frequency of transfer of antibiotic resistance markers was calculated for all conjugal matings as the number of transconjugants to the number of donor cells, as determined by viable counts.

Isolation and demonstration of plasmid DNA. Plasmid DNA was isolated from *E. faecalis* by modifications of the method of Macrina et al. (6). Cells were harvested from overnight cultures in 3 ml of MHB containing DL-threonine. Solid glycine was added to a concentration of 5%. The cells were centrifuged; resuspended in 1.5 ml of TES buffer (0.05 M NaCl, 0.05 M Tris base, 0.005 M disodium EDTA [pH 8]); and then treated sequentially with 0.3 ml of 25% sucrose in TE buffer (0.005 M disodium EDTA, 0.01 M Tris [pH 8.5]), 6 μl of RNase (Sigma) (5 mg/ml in 50 mM sodium acetate buffer, preheated at 80°C for 10 min), 70 μl of 1.5% lysozyme in TES buffer (pH 8), and 20 μl of 0.25 M EDTA. After incubation for 1 h at room temperature, the solution was cleared with 20 μl of 20% sodium dodecyl sulfate in 0.01 M Tris (pH 8), heated for 5 min at 60°C, treated with 65 μl of 5 M potassium acetate, and centrifuged. The supernatant containing plasmid DNA was further purified in cesium chloride-ethidium bromide by dye buoyant density gradient centrifugation (6) or immediately analyzed, by the method of Meyers et al. (8). in 10- to 20-μl volumes by electrophoresis on a standard vertical slab gel apparatus containing 0.7% agarose. Electrophoresis was performed at 10 mA (20 V) for 18 h.

Restriction enzyme analysis and DNA-DNA hybridization studies. Restriction enzyme digestion of purified plasmid DNA was done by the directions of the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Molecular size markers included *Hin*dIII and/or *Eco*RI digests of lambda DNA and the 1-kb ladder (Bethesda Research Laboratories, Gaithersburg, Md.). The 840-bp *Hin*dIII-*Xba*I fragment of recombinant plasmid pJM13 (kindly supplied by B. Murray [11, 23]) containing the β-lactamase gene was used to probe purified DNA from BL^+ *E. faecalis*. Probe DNA was labeled with [^{32}P]dCTP by nick translation by the directions of the manufacturer (New England Nuclear Corp., Boston, Mass.) and hybridized to target DNA under conditions of high stringency.

RESULTS

The effects of inoculum size on the antibiotic susceptibilities of 40 BL^+, AR isolates of *E. faecalis* are shown in Table 1. The MICs for 50 and 90 percent of strains increased for all β-lactamase-susceptible antibiotics when the inoculum of *E. faecalis* was increased from 10^5 to 10^7 CFU/ml. At a ratio of 2 to 1, ampicillin-sulbactam moderated, but did not eliminate, the inoculum effect. Sulbactam alone was inactive. Little inoculum effect was observed with imipenem, vancomycin, daptomycin, or ciprofloxacin, although tolerance to vancomycin was seen, with MBC/MIC ratios of ≥16 and ≥8 at inocula of 10^5 and 10^7 CFU/ml, respectively. Both an inoculum effect and tolerance were observed with teicoplanin. In contrast to the susceptibility pattern for BL^+, AR *E. faecalis* isolates, the following antibiotic susceptibility patterns (MIC/MBC for 90% of isolates tested, in micrograms per milliliter) were observed for 10 BL^-, AS isolates of *E. faecalis*: penicillin G, 4/8; ampicillin, 2/16; ampicillin-sulbactam, 2/8; imipenem, 2/2; gentamicin, 8/64; vancomycin, 2/≥32; teicoplanin, 0.25/≥32; daptomycin, 1/4; and ciprofloxacin, ≤1/4. Results obtained with an inoculum of 10^3 CFU/ml were exactly the same as the results obtained with an inoculum of 10^5 CFU/ml and, hence, were omitted from Table 1.

The bactericidal activities of antibiotics alone and in combination were determined in time-kill studies for 20 BL^+, AR isolates of *E. faecalis*. Bactericidal activity, when defined as a ≥3-$\log_{10}$-unit reduction of the initial inoculum, in CFU per milliliter, at 24 h was most marked with penicillin-sulbactam and ampicillin-sulbactam both in the frequency of significant killing and in the magnitude of the reduction in bacterial titers (Fig. 1 and Table 2). Among the other antibiotics tested, daptomycin was the most frequently bactericidal (Fig. 2 and Table 2). By the time-kill

TABLE 1. Comparative activities of various antibiotics against 40 BL^+, AR *E. faecalis* isolates

Antibiotic	Inoculum size (CFU/ml)	MIC_{50}/MBC_{50} (μg/ml)[a]	MIC_{90}/MBC_{90} (μg/ml)[b]	Range of MICs (μg/ml)
Penicillin	10^5	16/16	16/32	2–64
	10^7	≥128/≥128	≥128/≥128	32–≥128
Ampicillin	10^5	4/8	4/64	2–128
	10^7	≥128/≥128	≥128/≥128	32–≥128
Sulbactam	10^5	≥16/≥16	≥16/≥16	≥16
	10^7	≥16/≥16	≥16/≥16	≥16
Ampicillin-sulbactam[c]	10^5	1/1	2/2	0.5–2
	10^7	32/≥128	32/≥128	8–≥128
Imipenem	10^5	0.5/1	1/1	0.5–4
	10^7	1/1	1/4	1–4
Gentamicin	10^5	≥4,000/≥4,000	≥4,000/≥4,000	≥4,000
	10^7	≥4,000/≥4,000	≥4,000/≥4,000	≥4,000
Streptomycin	10^5	≥2,000/≥2,000	≥2,000/≥2,000	≥2,000
	10^7	≥2,000/≥2,000	≥2,000/≥2,000	≥2,000
Vancomycin	10^5	2/2	2/≥32	1–≥32
	10^7	4/≥32	4/≥32	4–≥32
Teicoplanin	10^5	0.25/≥32	0.25/3≥2	0.25–≥32
	10^7	≥32/≥32	≥32/≥32	2–≥32
Daptomycin	10^5	2/2	2/4	0.5–4
	10^7	4/8	4/16	2–16
Ciprofloxacin	10^5	1/4	1/8	0.5–8
	10^7	2/8	2/8	0.5–8
Rifampin	10^5	1/≥8	2/≥8	1–≥8
	10^7	≥8/≥8	≥8/≥8	8–≥8

[a] MIC_{50}/MBC_{50}, MIC/MBC for 50% of isolates tested.
[b] MIC_{90}/MBC_{90}, MIC/MBC for 90% of isolates tested.
[c] Ampicillin-sulbactam was used at a 2:1 ratio.

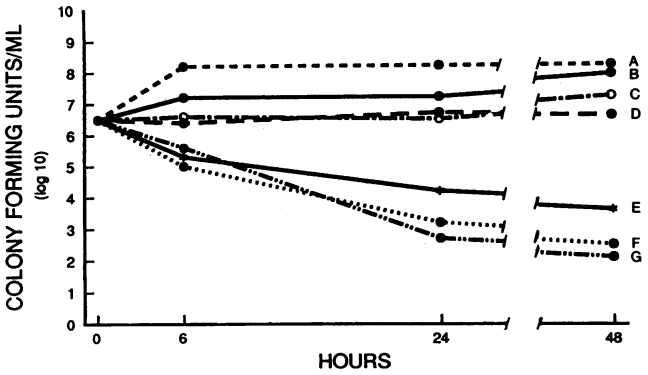

FIG. 1. Survival of BL^+, AR *E. faecalis* E47 in MHB (control, A) and MHB containing ampicillin, 4 μg/ml (B); penicillin G, 16 μg/ml (C); ampicillin, 16 μg/ml (D); penicillin G, 16 μg/ml, plus sulbactam, 8 μg/ml (E); ampicillin, 4 μg/ml, plus sulbactam, 2 μg/ml (F); and ampicillin, 16 μg/ml, plus sulbactam, 8 μg/ml (G). Concentrations of ampicillin or penicillin were equal to 1× or 4× the MICs for the test organism (ampicillin, 4 μg/ml; penicillin, 16 μg/ml; sulbactam, ≥8 μg/ml; and ampicillin-sulbactam, 1 μg [of ampicillin] per ml).

method, penicillin and ampicillin were inactive; however, enhanced killing was observed for 15 of 20 and 18 of 20 isolates with penicillin-sulbactam and ampicillin-sulbactam, respectively. Killing was neither enhanced nor antagonized with the addition of gentamicin (data not shown). With one exception, enhanced killing was not observed when vancomycin, teicoplanin, daptomycin, or ciprofloxacin was tested with ampicillin-sulbactam; killing for one isolate only was enhanced with the combination of ampicillin-sulbactam and ciprofloxacin when neither drug alone was active (Fig. 3).

Conjugal transfer of antibiotic resistance markers was observed for each of 20 BL^+, AR isolates of *E. faecalis*. Two transconjugants for each of 20 mating pairs with *E. faecalis* JH2-2 used as the recipient were randomly selected and evaluated for the transfer of β-lactamase production and aminoglycoside resistance. All transconjugants were resistant to gentamicin and streptomycin. Of 40 transconjugants, 38 produced β-lactamase by the nitrocefin disk method. All donors and transconjugants contained a 60- to 65-MDa plasmid, while *E. faecalis* JH2-2 and two BL^- isolates of *E. faecalis* (data not shown) lacked plasmids. The β-lactamase gene probe hybridized to a single 7.2-kb fragment of purified plasmid DNA from BL^+ isolates and transconjugants of *E. faecalis*.

TABLE 2. Activities of selected antibiotics by the time-kill method against 20 BL$^+$, AR *E. faecalis* isolates

Antibiotic(s)[a]	No. of isolates killed[b]/no. of isolates tested (%)	Mean $\log_{10}$ decrease (increase)[c] in CFU/ml at 24 h
Penicillin	0/20 (0)	(1)
Penicillin-sulbactam[d]	13/20 (65)	2.3
Ampicillin	0/20 (0)	(1.2)
Ampicillin-sulbactam[d]	13/20 (65)	2.8
Imipenem	0/20 (0)	0.2
Vancomycin	0/20 (0)	0.3
Teicoplanin	0/20 (0)	0.0
Daptomycin	8/20 (40)	2.1
Ciprofloxacin	2/20 (10)	1.4
Ciprofloxacin + ampicillin-sulbactam[d]	1/20 (5)	3.4

[a] Antibiotic concentrations equaled 1× or 4× the MIC for each isolate tested.
[b] See text; bactericidal activity was defined as a ≥3-$\log_{10}$-unit reduction in CFU per milliliter at 24 h compared with that in the initial inoculum.
[c] When compared with the CFU per milliliter in the initial inoculum.
[d] Penicillin and ampicillin were used at a ratio of 2:1 with sulbactam.

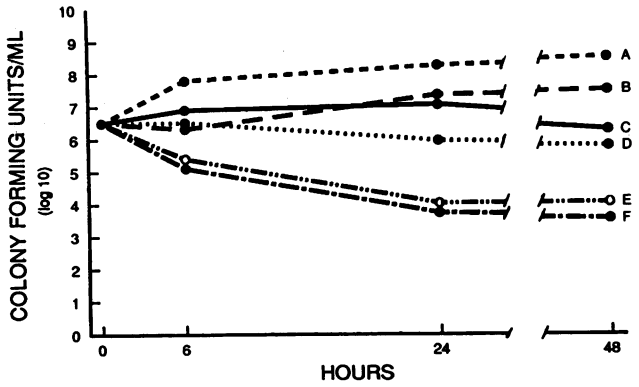

FIG. 2. Survival of BL$^+$, AR *E. faecalis* E47 in MHB (control, A) and MHB containing teicoplanin, 2 μg/ml (B); vancomycin, 4 μg/ml (C); vancomycin, 16 μg/ml (D); daptomycin, 4 μg/ml (E); and ciprofloxacin, 4 μg/ml (F). Concentrations of antibiotics were chosen to equal 1× or 4× the MICs for the test organism (vancomycin, 4 μg/ml; teicoplanin, 0.5 μg/ml; daptomycin, 1 μg/ml; and ciprofloxacin, 1 μg/ml).

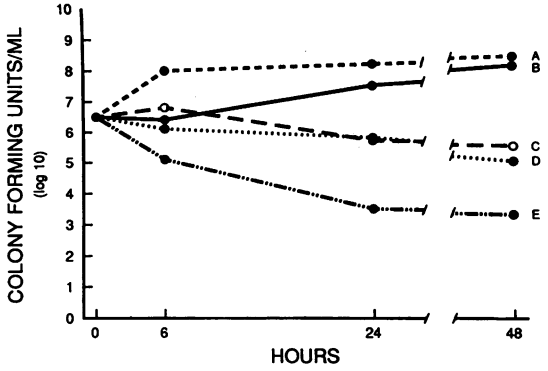

FIG. 3. Survival of a BL$^+$, AR *E. faecalis* isolate in MHB (control, A) and MHB containing ampicillin, 16 μg/ml (B); ciprofloxacin, 4 μg/ml (C); ampicillin, 16 μg/ml, plus sulbactam, 8 μg/ml (D); and ampicillin, 16 μg/ml, plus sulbactam, 8 μg/ml, plus ciprofloxacin, 4 μg/ml (E). MICs for the test organism were as follows: ampicillin, 4 μg/ml; ampicillin-sulbactam, 2 μg (of ampicillin) per ml; and ciprofloxacin, 1 μg/ml.

DISCUSSION

BL$^+$ *E. faecalis* isolates, most of which are also AR, have been reported in relatively few numbers since the initial description in 1983 (10). The gene for β-lactamase production in *E. faecalis* has been shown to be plasmid mediated, homologous to the gene for β-lactamase production in *Staphylococcus aureus*, and transferable (3, 10, 11, 20, 23). The presence in these strains of aminoglycoside resistance and the resultant elimination of synergism with certain β-lactam antibiotics creates a therapeutic dilemma. Although alternative regimens have been evaluated both in vitro and in vivo (1, 4, 12, 13, 15), the small number of isolates studied and the paucity of clinical reports preclude firm recommendations for therapy.

Our institution has been experiencing an ongoing problem with widespread colonization and infection with BL$^+$, AR *E. faecalis* isolates (21). Symptomatic infection has been frequent and occasionally life-threatening. The recovery of a large number of BL$^+$, AR isolates allowed us to evaluate the in vitro susceptibilities of organisms to single and combination antibiotic regimens, assess the effects of a β-lactamase inhibitor, and study the genetic basis of β-lactamase production and its transmissibility.

We believe that BL$^+$, AR *E. faecalis* isolates were endemic in our hospital well before their isolation, in part because some automated testing systems do not detect β-lactamase production by enterococci. We observed an inoculum effect for all β-lactam antibiotics tested, with the exception of imipenem, and noted that this effect was only partially reversed with the addition of a β-lactamase inhibitor, in contrast to the observations described in previous reports (12, 13, 15). Although our experiments were limited to a fixed dose combination of ampicillin-sulbactam, others have demonstrated a reversal of the inoculum effect by increasing the concentration of the β-lactamase inhibitor relative to the concentration of the β-lactam antibiotic (11).

Besides imipenem, no inoculum effect was observed with vancomycin, daptomycin, or ciprofloxacin, thereby confirming with a large number of BL$^+$, AR *E. faecalis* isolates what has been reported previously for a small number of isolates (9, 12, 13, 15). We observed not only a marked inoculum effect for teicoplanin but also a tolerance to the bactericidal effects of teicoplanin and vancomycin (4, 15). The bactericidal activities of vancomycin and teicoplanin have been shown in experimental rodent models of endocarditis caused by BL$^+$, AR *E. faecalis* isolates, but only two isolates have been studied (3, 4, 22). Despite the report of at least one cure with vancomycin of a patient with possible infective endocarditis caused by a BL$^+$, AR *E. faecalis* isolate (12) and the observed bactericidal effect against four strains in vitro (15), the results of our time-kill studies suggest that vancomycin or teicoplanin as a single-drug therapy for infections caused by BL$^+$, AR *E. faecalis* isolates should be used with caution unless bactericidal activity can be demonstrated.

For two isolates, MICs were ≥32 μg of vancomycin per ml (Table 1). Since both isolates were also resistant to teicoplanin, these organisms may be examples of inducible isolates with transferable high-level vancomycin resistance associated with the presence of a 39-kDa cytoplasmic membrane protein (18).

The most active antibiotics by the time-kill method were

penicillin G-sulbactam and ampicillin-sulbactam both in terms of the frequency with which bactericidal activity was observed and the magnitude of the reduction of bacterial titers. Murray et al. (11) and Patterson et al. (13) have previously shown the enhanced activity of β-lactam–β-lactamase inhibitor combinations in vitro. Ingerman et al. (4) and Hindes et al. (3) have demonstrated significant reductions in bacterial counts in and even sterilization of infected valvular tissue by using such combinations to treat experimental endocarditis caused by BL^+, AR *E. faecalis* isolates.

We noted bactericidal activity for only 8 of 20 isolates of BL^+, AR *E. faecalis* for daptomycin and 2 of 20 isolates for ciprofloxacin. The mean decrease, expressed as the $\log_{10}$ of the number of CFU per milliliter, at 24 h of 2.1 for daptomycin was within the 2- to 4.5-$\log_{10}$-unit decrease observed by Wanger and Murray (19). The bactericidal activity of daptomycin may be adversely affected by its high level of serum protein binding (1). We observed that the bactericidal activity of ampicillin-sulbactam (and penicillin G-sulbactam; data not shown) for one of seven isolates not killed by ampicillin-sulbactam was enhanced when ciprofloxacin was added, reducing the $\log_{10}$ CFU per milliliter by 3.4 at 24 h. To our knowledge, the observation of enhanced bactericidal activity of ciprofloxacin in combination with ampicillin-sulbactam (or penicillin G-sulbactam) against BL^+, AR *E. faecalis* isolates has not been reported previously.

We demonstrated and confirmed previous observations of the remarkable ease with which conjugal transfer of β-lactamase production and AR is achieved. Transfer of β-lactamase production was accomplished for all 20 BL^+ *E. faecalis* isolates; 38 of 40 transconjugants (95%) were β-lactamase producers. Cotransfer of high-level resistance to gentamicin and streptomycin was detected in all transconjugants. We attributed the transfer of these resistance markers to the presence in all BL^+ donors and transconjugants of a single 60- to 65-MDa conjugative plasmid species which was not found in several randomly selected BL^- *E. faecalis* and the two BL^- "transconjugants." Hybridization to the specific 840-bp β-lactamase gene probe confirmed the presence of the β-lactamase gene on the 60- to 65-MDa plasmid. This is in contrast to a recent report describing β-lactamase production and gentamicin resistance in isolates of *E. faecalis* whose genomic DNA but not plasmid DNA hybridized with the specific 840-bp β-lactamase gene probe and a probe derived from a bifunctional aminoglycoside resistance gene (17). The apparent chromosomal location of the β-lactamase gene and its low-frequency transmissibility suggest its incorporation into a transposable element.

The results of our in vitro susceptibility studies of the largest group of BL^+, AR *E. faecalis* isolates reported to date suggest alternative forms of therapy. In particular, ampicillin-sulbactam and, to a lesser extent, daptomycin seem to be reasonable choices for the empiric therapy of presumed enterococcal infections in hospitals in which BL^+, AR *E. faecalis* isolates are found. The use of any antibiotic regimen for infections caused by BL^+, AR *E. faecalis* isolates, particularly single-drug regimens, should ideally be supported by tests for bactericidal activity. Further clinical studies of alternative regimens are warranted.

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