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Comparative Evaluation of Penicillin, Ampicillin, and Imipenem MICs and Susceptibility Breakpoints for Vancomycin-Susceptible and Vancomycin-Resistant *Enterococcus faecalis* and *Enterococcus faecium*

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Although imipenem has in vitro activity against *Enterococcus faecalis* and Food and Drug Administration-approved indications for treatment of infections caused by this microorganism, there are no NCCLS guidelines for susceptibility testing of imipenem versus enterococci. Therefore, the in vitro activities of penicillin, ampicillin, imipenem, and vancomycin against 201 blood isolates of *E. faecalis* and 24 blood isolates of *Enterococcus faecium* were compared. The susceptibility of isolates to penicillin or ampicillin accurately predicted the in vitro activity of imipenem. Since the susceptibility of enterococci to imipenem can be predicted by the results obtained by testing of penicillin or ampicillin, testing of imipenem by clinical laboratories probably is not necessary.

Imipenem, the first widely used carbapenem antimicrobial agent, was shown in early studies to have good in vitro activity against *Enterococcus faecalis* (1, 4, 5, 9, 10) but little activity against *Enterococcus faecium* (2). However, present NCCLS-approved standards for in vitro susceptibility testing (6, 7) provide no guidance for testing imipenem versus enterococci, nor are there statements that in vitro susceptibility results for other antimicrobial agents can predict the in vitro activity of imipenem against these bacteria. Nevertheless, clinicians and microbiologists attending a recent meeting of the NCCLS Subcommittee on Antimicrobial Susceptibility Testing (June 1999) agreed that penicillin and/or ampicillin treatment likely would predict the activity of imipenem (M. P. Weinstein, personal observation). In the absence of systematically gathered and published data, however, the question remains open. Therefore, 225 isolates from patients with enterococcal bacteremia were tested against imipenem, penicillin, ampicillin, and vancomycin to determine the in vitro activity of each agent as well as the degree to which each drug predicted the in vitro activity of imipenem.

Enterococcal isolates causing bacteremia in patients hospitalized at Robert Wood Johnson University Hospital from July 1997 through October 1999 were tested. These included 201 *E. faecalis* (24 vancomycin-resistant) and 24 *E. faecium* (19 vancomycin-resistant) isolates. All isolates were identified in the Robert Wood Johnson University Hospital Clinical Microbiology Laboratory using dried-overnight (conventional) gram-positive combination panels in the MicroScan WalkAway 96 Instrument (Dade MicroScan, Inc., West Sacramento, Calif.). Species identification of strains with unusual susceptibility patterns (e.g., vancomycin-intermediate *E. faecalis* and ampicillin-susceptible *E. faecium*) was confirmed by conventional microbiological testing (3). Prior to testing, isolates were thawed and subcultured twice to ensure purity and viability.

Each isolate was tested versus penicillin, ampicillin, imipenem, and vancomycin. Solutions of all antimicrobials were prepared from standard powders of known potencies obtained either from the manufacturer of the compound or from a commercial source (Sigma, St. Louis, Mo.). MICs were determined in duplicate by the microdilution method of the NCCLS using cation-adjusted Mueller-Hinton broth (7).

Of the 201 *E. faecalis* strains tested, 175 were susceptible to vancomycin (MIC $\leq$ 4 μg/ml), 2 were intermediate (MIC = 8 μg/ml for both strains), and 24 were resistant (MIC $\geq$ 32 μg/ml). The MIC ranges, MICs at which 50% of the isolates tested were inhibited (MIC_{50}s), and MIC_{90}s of penicillin, ampicillin, and imipenem were comparable for vancomycin-susceptible and vancomycin-resistant isolates (Table 1). The MIC_{90}s of all three agents were in the susceptible range even for the vancomycin-resistant isolates. Of the 24 *E. faecium* strains tested, 5 were susceptible to vancomycin and 19 were resistant to vancomycin. The penicillin, ampicillin, and imipenem MIC ranges for the five vancomycin-susceptible strains are shown in Table 1. Two of the five strains were susceptible to penicillin and ampicillin, whereas MICs for three strains were of $\geq$64 μg/ml. For the 19 vancomycin-resistant strains, MIC ranges and MIC_{50}s and MIC_{90}s of all three antimicrobials were >64 μg/ml (Table 1).

Using the present published NCCLS breakpoints for penicillin and ampicillin versus enterococci (7) (susceptible, $\leq$8 μg/ml; resistant, $\geq$16 μg/ml) and the breakpoints for imipenem published in the manufacturer's package insert approved by the Food and Drug Administration (FDA) (susceptible, $\leq$ 4 μg/ml; intermediate, 8 μg/ml; resistant, $\geq$16 μg/ml), the ability of penicillin and ampicillin MICs to predict in vitro susceptibility of enterococci versus imipenem was assessed. As shown in Table 2, of the 201 *E. faecalis* strains tested, penicillin results

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TABLE 1. Susceptibility of vancomycin-susceptible and vancomycin-resistant enterococci to penicillin, ampicillin, and imipenem[a]

Microorganism (*n*)	Antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Vancomycin-susceptible *E. faecalis* (175)	Penicillin	0.5–16	4	4
	Ampicillin	0.25–4	1	2
	Imipenem	0.06–4	1	2
Vancomycin-resistant *E. faecalis* (24)	Penicillin	2–4	4	4
	Ampicillin	0.5–4	1	2
	Imipenem	0.5–2	1	2
Vancomycin-susceptible *E. faecium* (5)	Penicillin	2–>64		
	Ampicillin	1–>64		
	Imipenem	0.5–>64		
Vancomycin-resistant *E. faecium* (19)	Penicillin	>64	>64	>64
	Ampicillin	≥64	>64	>64
	Imipenem	≥64	>64	>64

[a] Two *E. faecalis* strains were intermediately susceptible to vancomycin (MIC = 8 μg/ml). Empty cells indicate that the MIC_{50} and MIC_{90} were not calculated ($n < 10$).

correctly predicted imipenem results for 200 (99.5%) strains, and ampicillin results correctly predicted the results for imipenem for all 201 strains. For the 24 *E. faecium* strains, the results for penicillin and ampicillin were identical. Of two penicillin- and ampicillin-susceptible strains, one was susceptible to imipenem and one was intermediate (Table 2). All 22 strains that were resistant to penicillin and ampicillin were also resistant to imipenem.

The results of this study confirm the widely held but poorly documented belief that the in vitro activity of penicillin and ampicillin versus *E. faecalis* and *E. faecium* accurately predicts that of imipenem. The importance of this information lies in the fact that enterococci often are potential pathogens of mixed infections for which a broad-spectrum antimicrobial agent such as imipenem has a therapeutic role. Imipenem currently has FDA indications for use in intra-abdominal infections, skin and skin structure infections, and gynecologic infections caused by *E. faecalis* but not by *E. faecium*. The drug also is used in some institutions as monotherapy for patients with neutropenic fever.

Presently, there are no NCCLS susceptibility testing guidelines for imipenem against enterococci (6, 7) and, based on the data from this study, specific testing guidelines for imipenem do not appear to be needed. However, several limitations of the data make firm conclusions from this report problematic. First, all of the microorganisms tested came from a single institution. Second, a relatively small number of *E. faecium* strains were tested. Although it is possible that these strains might represent only a few clones, prior work from our institution has shown our *E. faecium* strains to be polyclonal (H. Soliman, K. L. Joho, K. Damerau, R. Kuk, M. P. Weinstein, and J. F. John, Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993, abstr. A-80, p. 15, 1993). Third, no species other than *E. faecalis* and *E. faecium* were included.

To address these limitations, it will be necessary to study additional *E. faecalis* and *E. faecium* strains from other geographic regions and, if possible, to include less common enterococcal species, such as *Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus raffinosus,* and *Enterococcus avium*, thereby meeting the suggested criteria of the NCCLS (8). If the initial results from the present study are confirmed, microbiology laboratories and clinicians will benefit from a therapeutic note in the NCCLS guidelines and tables indicating that the in vitro results obtained for penicillin or ampicillin will accurately predict the in vitro susceptibility of imipenem.

TABLE 2. Correlation of penicillin and ampicillin NCCLS susceptibility breakpoints for enterococci with imipenem FDA susceptibility breakpoints[a]

Strain[b]	Breakpoint for penicillin or ampicillin (μg/ml)	No. of strains correlating with imipenem breakpoint (μg/ml)		
		≤4	8	≥16
E. faecalis	Penicillin (≤8)	200	0	0
	Penicillin (≥16)	1	0	0
	Ampicillin (≤8)	201	0	0
	Ampicillin (≥16)	0	0	0
E. faecium	Penicillin (≤8)	1	1	0
	Penicillin (≥16)	0	0	22
	Ampicillin (≤8)	1	1	0
	Ampicillin (≥16)	0	0	22

[a] NCCLS breakpoints (in micrograms per milliliter) for penicillin and ampicillin: ≤8, susceptible; ≥16, resistant. FDA breakpoints (in micrograms per milliliter) for imipenem: ≤4, susceptible; 8, intermediate; ≥16, resistant.

[b] For *E. faecalis*, $n = 201$, including 24 vancomycin-resistant strains. For *E. faecium*, $n = 24$, including 19 vancomycin-resistant strains.

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