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Antibacterial Activity of Fortimicin A Compared with Those of Five Other Aminoglycosides, and Factors Affecting Susceptibility Tests

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Fortimicin A, a pseudodisaccharide aminoglycoside, was found to have broad-spectrum activity against most clinically important aerobic and facultatively anaerobic bacteria, except *Pseudomonas aeruginosa*, some other *Pseudomonas* species, and streptococci. It was comparable to amikacin in its level of activity (minimum inhibitory concentrations) and spectrum of activity (except for the lack of activity on *P. aeruginosa*). Fortimicin A was bactericidal and was affected by cations when tested against *P. aeruginosa*. Minimum inhibitory concentrations were affected by the inoculum used in the susceptibility test. The drug was resistant to most aminoglycoside-inactivating enzymes, but probably is not active against permeability mutants.

Fortimicin A is the most active member of the fortimicin complex of aminoglycosidic antibiotics produced by *Micromonospora olivoastrospora* (6, 8). It is a pseudodisaccharide-incorporating fortamine, a novel aminocyclitol (3). It possesses a broad spectrum of activity against gram-positive and gram-negative bacteria, but is less active against *Pseudomonas aeruginosa* than are some of the other newer aminoglycosides (4-6). It is resistant to the activity of some of the aminoglycoside-inactivating enzymes (4, 9).

In this study we compared the antibacterial activity of fortimicin A with those of five other aminoglycosides (amikacin, gentamicin, netilmicin, sisomicin, and tobramycin) by testing them against a group of bacteria selected to represent a wide variety of genera and species with various susceptibility patterns and mechanisms of resistance. We also determined the bactericidal activity and the effect of changes in cation content of the medium and in inocula on the in vitro activity of the drug.

MATERIALS AND METHODS

Protocol. The primary investigators developed a protocol for the collaborative investigation of the drug in three laboratories (10). The in vitro activity of the drug against the organism was determined in two laboratories, Center for Disease Control, Atlanta, Ga., and University of California, Davis. Comparable results have been repeatedly obtained in these two laboratories, but to ensure this interlaboratory agreement, some strains of each genus were tested in both laboratories (only one set of data was reported for these strains).

The effect of inoculum changes on the minimum inhibitory concentrations (MICs) for some of these strains was determined in a third laboratory (Kaiser Foundation Hospital Laboratories, Portland, Ore.).

Antibiotics. Antibiotic powders suitable for antimicrobial susceptibility tests were obtained as follows: fortimicin A from Abbott Laboratories, Chicago, Ill.; gentamicin, sisomicin, and netilmicin from Schering Corp., Bloomfield, N.J.; amikacin from Bristol Laboratories, Syracuse, N.Y.; and tobramycin from Lilly Laboratories, Indianapolis, Ind.

Bacteria. This collection of organisms was assembled for the purpose of challenging this aminoglycoside with a wide range of species and of resistance patterns without regard to usual distribution, as described previously (10). Most of the organisms were selected from recent clinical isolates in the laboratories participating in these studies. The culture collection was supplemented with stock cultures of uncommon isolates, such as β-lactamase-producing *Neisseria gonorrhoeae* and *Haemophilus influenzae*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas* species other than *P. aeruginosa*. In addition, tests were performed with a collection of 25 strains known to produce certain aminoglycoside-inactivating enzymes or to be permeability mutants. The effect of inoculum variation and the comparison of MICs with minimum lethal concentrations (MLCs) were studied with 70 strains selected from the study organisms that yielded results in a measurable range.

Antimicrobial susceptibility tests. MICs were determined by the broth microdilution method (10, 11). The trays were prepared commercially (Prepared Media Laboratories, Portland, Ore.) by using single lots of Mueller-Hinton broth and single lots of each antimicrobic. Fortimicin A was tested in unsupplemented broth and in cation-supplemented broth (calcium, 50 mg/liter, and magnesium, 25 mg/liter). The other aminoglycosides were tested only in the supple-

mented broth. The microdilution trays were shipped to the participating laboratories in the frozen state and stored at −70°C until used. When needed, trays were removed from the freezer and left at room temperature to permit the broth to thaw, and then the tests were performed.

The inocula were prepared from actively growing broth cultures, which were adjusted to match the turbidity of a 0.5 McFarland standard (7), and were diluted 1:50 in sterile distilled water containing 0.02% Tween 80. Disposable inoculators were used to inoculate the microdilution trays with these adjusted inocula; the final inoculum was approximately 10^5 colony-forming units (CFU) per ml.

In the tests with *Streptococcus pneumoniae* and *S. pyogenes*, the Mueller-Hinton broth was supplemented with 5% lysed rabbit blood by preparing the inoculum in 10% lysed rabbit blood and then adding an equal amount to the broth in each well (0.1 ml).

In the same manner, 1% Fildes reagent was added to the broth for tests with *H. influenzae*. *N. gonorrhoeae* isolates were tested by agar dilution as described previously (10, 11).

The trays and plates were incubated at 35°C for 18 to 24 h. The MIC was read as the lowest concentration of drug that prevented macroscopically visible growth.

The MLC was determined on selected organisms by subculturing approximately 5 μl from each well of the MIC tray to Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 5% sheep blood. The transfer was made with a multiple-point inoculator. After 48 h of incubation, the MLC was read as the lowest concentration of drug yielding no growth on subculture (or 99.9% kill).

For the inoculum studies, the organisms were diluted so that the final inoculum in the wells was 10^3, 10^5, or 10^7 CFU/ml. The MICs were determined as described above.

RESULTS AND DISCUSSION

The ranges of the MICs and the MICs needed to inhibit 50 and 90% (MIC_{50}, MIC_{90}) of the *Enterobacteriaceae* strains are shown in Table 1. Fortimicin is active against most of these strains, the exceptions being occasional strains of indole-positive *Proteus* (including the new genera *Morganella morganii* and *Providencia rettgeri*) and *Providencia stuartii* (1). The same general pattern of activity was obtained with the other five aminoglycosides. The level of activity parallels that of amikacin and is, in general, less than those of gentamicin, netilmicin, sisomicin, and tobramycin (on a weight basis), but for the *P. stuartii* strains, fortimicin and amikacin were generally more active. Sisomicin was the most active aminoglycoside against members of the *Enterobacteriaceae*.

The ranges and the MIC_{50} and MIC_{90} values for the nonfermentative gram-negative bacilli are shown in Table 2. Although occasional strains of *P. aeruginosa* were susceptible to fortimicin, most were not, and its activity was less than those of the other five aminoglycosides. The activity of fortimicin against the other species of *Pseudomonas* appeared to be species specific: *P. cepacia* and *P. acidovorans* were resistant; *P. fluorescens*, *P. putida*, and *P. stutzeri* were susceptible. The same pattern was obtained for the other five aminoglycosides except for *P. maltophilia*, for which the MICs were quite variable. Most of the *Acinetobacter* strains were susceptible to all of the aminoglycosides. However, these conclusions about the *Pseudomonas* species other than *P. aeruginosa* must be viewed with caution because of the small numbers of each species tested.

The ranges and MIC_{50} and MIC_{90} values for methicillin-susceptible and -resistant *S. aureus*, streptococci, and β-lactamase-negative and -positive strains of *N. gonorrhoeae* and *H. influenzae* are shown in Table 3. Most of the staphylococci were susceptible to all of these aminoglycosides, with fortimicin and amikacin MICs being higher than those of the other four drugs. There was little difference between the susceptibility patterns of the methicillin-susceptible and the methicillin-resistant strains. Although occasional strains of streptococci were susceptible to these aminoglycosides, most were resistant.

Fortimicin A and the other five aminoglycosides were active against the gonococci and the *H. influenzae* strains. The differences in MICs between the β-lactamase-negative and -positive strains were negligible. Fortimicin A and amikacin MICs were similar and slightly higher than those of the other four aminoglycosides.

These data show that fortimicin A closely resembles amikacin in its spectrum and level of activity, with the exception that it is much less active against *P. aeruginosa* strains. Fortimicin A MICs are, therefore, generally higher than MICs of gentamicin, netilmicin, sisomicin, and tobramycin. These results are in general agreement with previous reports (4, 5). We found in this study, as was found in the collaborative study of Jones et al. (5), that fortimicin A and amikacin were more active against *P. stuartii* than was gentamicin. We also found these drugs to be more active than netilmicin, sisomicin, and tobramycin against *P. stuartii*. However, we did not find fortimicin A and amikacin to be more active in vitro against *Serratia marcescens* than gentamicin, as did Jones et al. (5). Although fortimicin A and amikacin were active against *Serratia* species, the MICs were slightly higher than those of gentamicin, netilmicin, sisomicin, and tobramycin. The differences in the fortimicin A and gentamicin MICs for the *S. marcescens* strains in the study by Jones et al. were due to fortimicin-susceptible, gentamicin-resistant strains from one of the collaborating institutions (5). Therefore, the differences for *Serratia* found

TABLE 1. *MICs of fortimicin A and five other aminoglycosides for 181 strains of Enterobacteriaceae*[a]

Organism (no. of strains)	Drug	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
E. coli (25)	Fortimicin A	1.0–8.0	4	4
	Amikacin	1.0–8.0	2	4
	Gentamicin	≤0.25–2.0	1	1
	Netilmicin	≤0.25–2.0	0.5	1
	Sisomicin	≤0.25–1.0	0.5	1
	Tobramycin	0.5–2.0	1	1
Enterobacter (25) [includes *E. cloacae* (10), *E. aerogenes* (10), *E. agglomerans* (5)]	Fortimicin A	0.5–4.0	2	4
	Amikacin	0.5–4.0	1	4
	Gentamicin	≤0.25–2.0	0.5	1
	Netilmicin	≤0.25–0.5	0.5	0.5
	Sisomicin	≤0.25–1.0	0.5	0.5
	Tobramycin	≤0.25–2.0	0.5	1
K. pneumoniae (25)	Fortimicin A	1.0–8.0	2	4
	Amikacin	0.5–4.0	1	2
	Gentamicin	≤0.25–8.0	0.5	1
	Netilmicin	≤0.25–1.0	0.5	0.5
	Sisomicin	≤0.25–2.0	0.5	0.5
	Tobramycin	≤0.25–16.0	0.5	1
P. mirabilis (26)	Fortimicin A	2.0–16.0	4	8
	Amikacin	0.5–16.0	2	8
	Gentamicin	≤0.25–4.0	1	2
	Netilmicin	≤0.25–4.0	1	2
	Sisomicin	≤0.25–2.0	0.5	1
	Tobramycin	≤0.25–2.0	0.5	1
Proteus, indole positive (30) [includes *M. morganii* (10), *P. vulgaris* (10), *P. rettgeri* (10)]	Fortimicin A	≤0.25–128.0	2	8
	Amikacin	0.5–8.0	1	2
	Gentamicin	≤0.25–64.0	1	8
	Netilmicin	≤0.25–64.0	1	16
	Sisomicin	≤0.25–32.0	0.5	4
	Tobramycin	≤0.25–32.0	1	4
P. stuartii (25)	Fortimicin A	≤0.5–128.0	2	8
	Amikacin	≤0.25–128.0	1	4
	Gentamicin	≤0.25–128.0	8	32
	Netilmicin	≤0.25–>128.0	8	32
	Sisomicin	≤0.25–64.0	4	8
	Tobramycin	≤0.25–128.0	4	16
S. marcescens (25)	Fortimicin A	1.0–8.0	4	8
	Amikacin	1.0–16.0	2	8
	Gentamicin	0.5–64.0	1	16
	Netilmicin	0.5–16.0	2	4
	Sisomicin	≤0.25–32.0	0.5	8
	Tobramycin	1.0–128.0	4	128

[a] MICs were determined in cation-supplemented Mueller-Hinton broth.

in their study compared with our study were for gentamicin and not for fortimicin. We also found most *S. pneumoniae* strains to be resistant to fortimicin, in contrast to the report of Jones et al. (5).

For all strains tested, the most active drug in vitro (i.e., MIC) was sisomicin, followed by netilmicin, tobramycin, gentamicin, amikacin, and fortimicin. With respect to clinical significance, however, absolute drug activity must be balanced against the concentrations which can be attained in serum and tissue and can be maintained safely. Since these concentrations are significantly higher for fortimicin and amikacin, these compounds may be as clinically active as the aminoglycosides with lower MICs.

The effect of cation concentration on the in vitro activity of fortimicin A was seen principally with *P. aeruginosa*, with MICs obtained in broth containing physiological levels of calcium and magnesium being about 16-fold higher than those obtained in unsupplemented broth. For-

TABLE 2. *MICs of fortimicin A and five other aminoglycosides for 125 strains of nonfermentative gram-negative bacilli*[a]

Organism (no. of strains)	Drug	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
P. aeruginosa (80)	Fortimicin A	1.0->128	128	>128
	Amikacin	0.5->128	8	32
	Gentamicin	8.0->128	8	64
	Netilmicin	≤0.25->128	16	64
	Sisomicin	≤0.25->128	4	16
	Tobramycin	≤0.25->128	2	32
P. stutzeri (10)	Fortimicin A	0.5-2.0	2	4
	Amikacin	0.5-2.0	1	2
	Gentamicin	≤0.25-0.5	0.5	0.5
	Netilmicin	≤0.25-0.5	≤0.25	0.5
	Sisomicin	≤0.25-0.5	≤0.25	0.5
	Tobramycin	≤0.25-1.0	0.5	0.5
P. cepacia (4)	Fortimicin A	>128	—[b]	—
	Amikacin	32.0->128		
	Gentamicin	64->128		
	Netilmicin	128->128		
	Sisomicin	32->128		
	Tobramycin	32->128		
P. maltophilia (3)	Fortimicin A	8.0->128	—	—
	Amikacin	8.0->128		
	Gentamicin	1.0->128		
	Netilmicin	1.0->128		
	Sisomicin	1.0->128		
	Tobramycin	2.0->128		
P. acidovorans (3)	Fortimicin A	>128	—	—
	Amikacin	64-128		
	Gentamicin	128->128		
	Netilmicin	>128		
	Sisomicin	64-128		
	Tobramycin	32-64		
P. fluorescens (6)	Fortimicin A	0.5-1.0	—	—
	Amikacin	≤0.25-0.5		
	Gentamicin	≤0.25-16		
	Netilmicin	≤0.25-0.5		
	Sisomicin	≤0.25-8.0		
	Tobramycin	≤0.25-16		
P. putida (5)	Fortimicin A	1.0-4.0	—	—
	Amikacin	1.0-4.0		
	Gentamicin	0.5-4.0		
	Netilmicin	1.0-4.0		
	Sisomicin	≤0.25-1.0		
	Tobramycin	≤0.25-1.0		
Acinetobacter (14)	Fortimicin A	1.0-128	4	16
	Amikacin	1.0-32	2	4
	Gentamicin	0.5-1.0	0.5	4
	Netilmicin	0.5->128	2	32
	Sisomicin	0.5-128	0.5	4
	Tobramycin	0.5-64	1	8

[a] MICs were determined in cation-supplemented Mueller-Hinton broth.
[b] —, Number of organisms too small to determine these MICs.

timicin A MICs for *P. fluorescens* and *P. putida* were also four- to eightfold higher than in the supplemented broth, but the number of strains is too small to draw definite conclusions. *P. cepacia*, *P. maltophilia*, and *P. acidovorans* were too resistant to determine the effects of

TABLE 3. *MICs of fortimicin A and five other aminoglycosides for 199 strains of staphylococci, streptococci, gonococci, and H. influenzae*[a]

Organism (no. of strains)	Drug	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
S. aureus methicillin susceptible (50)	Fortimicin A	≤0.25–128	1	2
	Amikacin	≤0.25–2.0	1	2
	Gentamicin	≤0.25–64	≤0.25	0.5
	Netilmicin	≤0.25–2.0	≤0.25	≤0.25
	Sisomicin	≤0.25–16	≤0.25	≤0.25
	Tobramycin	≤0.25–16	≤0.25	0.5
S. aureus methicillin resistant (10)	Fortimicin A	≤0.25–2.0	1	2
	Amikacin	≤0.25–2.0	1	2
	Gentamicin	≤0.25–0.5	≤0.25	0.5
	Netilmicin	≤0.25–0.5	<0.25	0.5
	Sisomicin	≤0.25–0.5	≤0.25	≤0.25
	Tobramycin	≤0.25–8.0	≤0.25	1
S. pyogenes (20)	Fortimicin A	8.0–64	32	64
	Amikacin	8.0–64	64	>64
	Gentamicin	2.0–32	16	32
	Netilmicin	2.0–16	16	16
	Sisomicin	2.0–16	8	16
	Tobramycin	16–64	32	64
S. pneumoniae (19)	Fortimicin A	4.0–64	32	32
	Amikacin	8.0–>64	64	>64
	Gentamicin	1.0–32	16	32
	Netilmicin	2.0–16	8	16
	Sisomicin	1.0–16	8	16
	Tobramycin	8.0–64	16	64
S. faecalis (64)	Fortimicin A	16–>64	32	128
	Amikacin	32–>64	>128	>128
	Gentamicin	4.0–>64	16	32
	Netilmicin	8.0–32	16	32
	Sisomicin	8.0–32	16	32
	Tobramycin	16–32	64	>64
N. gonorrhoeae, β-lactamase negative (24)	Fortimicin A	4.0–8.0[b]	8	8
	Amikacin	4.0–16.0	8	16
	Gentamicin	1.0–2.0	2	2
	Netilmicin	1.0–2.0	1	2
	Sisomicin	1.0–2.0	2	2
	Tobramycin	2.0–4.0	4	4
N. gonorrhoeae, β-lactamase positive (26)	Fortimicin A	2.0–16[b]	8	8
	Amikacin	4.0–16	8	16
	Gentamicin	1.0–4.0	2	2
	Netilmicin	0.5–2.0	1	2
	Sisomicin	1.0–4.0	1	2
	Tobramycin	2.0–4.0	2	4
H. influenzae, β-lactamase negative (20)	Fortimicin A	1.0–4.0	4	4
	Amikacin	2.0–8.0	4	8
	Gentamicin	0.5–2.0	1	2
	Netilmicin	≤0.25–1.0	0.5	1
	Sisomicin	≤0.25–1.0	1	1
	Tobramycin	0.5–2.0	1	2
H. influenzae β-lactamase positive (20)	Fortimicin A	2.0–4.0	2	4
	Amikacin	2.0–8.0	2	4
	Gentamicin	1.0–2.0	1	2
	Netilmicin	0.5–1.0	0.5	1
	Sisomicin	0.5–1.0	0.5	1
	Tobramycin	1.0–2.0	1	2

[a] MICs were determined in cation-supplemented Mueller-Hinton broth.
[b] Agar dilution MICs (see text for method).

cations. The fortimicin A MICs obtained for all of the other strains were very similar for both media.

We have previously recommended that cation-supplemented Mueller-Hinton broth be used in microdilution tests to obtain clinically useful susceptibility data with *P. aeruginosa* and aminoglycosides (11). This recommendation is now made for fortimicin A also. In this study, the fortimicin A MICs obtained with cation-supplemented broth are comparable to those obtained with cation-supplemented broth and with agar dilution in two previous studies (4, 5). Mueller-Hinton agar usually (but not always) contains cations in concentrations similar to those in supplemented broth. The comparison of aminoglycoside MICs made above were with the fortimicin A MICs obtained in cation-supplemented broth.

A comparison of the MLCs and MICs of fortimicin was made for some of the strains used in this study. Of the 420 determinations made with 70 selected strains and the 6 aminoglycosides, 83% were the same and in 13% the MLC was one dilution higher, in 3% it was two dilutions higher, and in 1% it was three dilutions higher. No fortimicin MLC was more than two dilutions higher. The two-dilution differences for fortimicin were found with *S. aureus*, indole-positive *Proteus*, and *S. marcescens*. In a previous study with the new aminoglycoside Sch 21420, we found that *S. marcescens* was more likely to have higher MLC/MIC ratios (10). We concluded that the fortimicin A MLCs and MICs are essentially the same for most strains and that fortimicin A is usually bactericidal at the inhibitory level.

The effect of inoculum concentration on the fortimicin A MICs of some of the organisms in this study is shown in Table 4. The mode MICs obtained with inocula of 10^3 and 10^5 CFU/ml were generally the same or within one log dilution (the major exception was the netilmicin MICs for *Proteus* spp., which were ≤0.25 and 2 μg/ml). With inocula of 10^7 CFU/ml, the effects on MICs were more pronounced, in many cases being four- to eightfold those with 10^3 and 10^5 CFU/ml.

We conclude that there is an inoculum effect in MIC tests performed with fortimicin A. We recommend a standard inoculum of 10^5 CFU/ml in microdilution tests. The tests in these studies, except for those with inoculum, were performed with 10^5 CFU/ml.

The activity of fortimicin A on strains that produce aminoglycoside-inactivating enzymes (2) or that are permeability mutants is shown in Table 5. The activity more closely resembles that of amikacin, except for the *P. aeruginosa* strains. Except for one strain of *P. aeruginosa* with an acetylation enzyme [AAC(6')-I], the MICs of amikacin were lower than those of

TABLE 4. *MIC modes obtained for seven groups of bacteria when inoculum concentrations were increased from 10^3 to 10^7 CFU/ml*

Organism (no. of strains)	MIC (μg/ml)																	
	Fortimicin A			Amikacin			Gentamicin			Netilmicin			Sisomicin			Tobramycin		
	3[a]	5	7	3	5	7	3	5	7	3	5	7	3	5	7	3	5	7
S. marcescens (10)	2	2	12[b]	1.5	2	16	0.5	1	2	0.5	1	4, 16	0.5	0.5	2	0.75[b]	1	8
Enterobacter spp. (10)[c]	2	2	8	1	1	4	0.5	0.5	2	0.5	0.5	1	≤0.25	≤0.25	1	0.5	0.5	2
E. coli (10)	2	4	32	1	4	32	0.5	1	8	0.5	1	4	0.5	0.75[b]	4	0.5	1	16
K. pneumoniae (10)	2	4	8	1	2	4	0.5	0.5	4	≤0.25, 0.5	0.5	1	≤0.25	0.5	1	0.5	0.5	2
S. aureus (10)	1	1	6[b]	0.5	1	6[b]	≤0.25	≤0.25	2	≤0.25	≤0.25	1	≤0.25	≤0.25	1	≤0.25	≤0.25	1.5[b]
Proteus spp. (10)[d]	2	2	16	0.5	0.5	8	≤0.25	0.5, 2	4	≤0.25	2	2, 16	≤0.25, 1	0.5, 2	2	≤0.25, 2	2	4
P. aeruginosa (10)	32	64	128	8	16	8	4	4, 16	16	8	16	16	2	4, 16	8	0.5, 2	8	8

[a] Inoculum concentration in $\log_{10}$-scale CFU per milliliter.

[b] When there are two equal populations at adjacent dilution intervals, the mode is listed as a concentration half-way between the two values except when the MICs were not on scale.

[c] Includes *E. cloacae* (5) and *E. aerogenes* (5).

[d] Includes *Providencia rettgeri* (4), *Morganella morganii* (4), and *Proteus vulgaris* (2).

TABLE 5. *Geometric mean MICs of fortimicin A and five aminoglycosides against 25 strains possessing known resistance mechanisms[a]; MICs were determined in cation-supplemented Mueller-Hinton broth.*

Resistance mechanism(s)	Bacterial species (no. of strains)	Geometric mean MIC (μg/ml)					
		Fortimicin A	Amikacin	Gentamicin	Netilmicin	Sisomicin	Tobramycin
APH(3')-I[a]	*E. coli* (1)	8	4	2	2	1	4
APH(3')-IV	*S. aureus* (3)	6	29.3	0.7	0.7	0.8	4.3
ANT(2")	*P. aeruginosa* (1)	64	8	>128	16	128	64
ANT(2")	*S. liquefaciens* (1) *E. coli* (1)	4.5	6	96	1.5	40	64
ANT(4')	*S. aureus* (1)	4	16	1	0.5	0.5	>128
AAC(3)-I	*P. aeruginosa* (2)	128	24	>128	>128	>128	4
AAC(3)-II	*P. aeruginosa* (1)	>128	16	>128	64	>128	>128
AAC(3)-II	*S. marcescens* (1) *K. pneumoniae* (1)	6	6	>128	20	48	10
AAC(3)-III	*E. coli* (1)	2	2	64	16	64	16
AAC(6')-I	*P. aeruginosa* (1)	64	128	32	>128	64	128
AAC(6')-I	*E. coli* (1) *S. marcescens* (1) *Moraxella* sp. (1)	9.3	32.7	44.7	107	28	74.7
AAC(6')-II	*P. aeruginosa* (1)	128	32	>128	>128	>128	>128
APH(3')-I + AAC(2')	*P. rettgeri* (1)	4	8	32	64	32	16
APH(3')-II + AAC(6')-I	*S. marcescens* (1)	16	16	16	16	4	64
APH(3')-II + ANT(2")	*K. pneumoniae* (1)	4	2	64	0.5	32	32
PERM[b]	*P. aeruginosa* (2)	>128	48	48	96	24	12
PERM	*E. coli* (1)	128	64	32	64	32	16

[a] See reference 2 for classification of enzymes.
[b] PERM, Permeability mutant.

fortimicin, including permeability mutants. Some organisms, e.g., *S. aureus* with a phosphorylation enzyme [APH(3')-IV] and an adenylation enzyme [ANT(4')] and the strains with AAC(6')-I enzymes, were more susceptible to fortimicin than to amikacin. Overall, fortimicin A and amikacin were about equal in activity against these strains and more active than the other four aminoglycosides.

Fortimicin has been reported to be a poor substrate for common inactivating enzymes (4, 6). However, Nara et al. (6) showed that fortimicin was acetylated by an *Escherichia coli* strain that produced enzyme AAC(3)-I. Jones et al. (5) reported that staphylococci producing both APH(2") and AAC(6') were resistant to fortimicin. Even though the data with *P. aeruginosa* are more difficult to interpret because of the intrinsic resistance of these strains to fortimicin A, they do confirm the previous supposition that fortimicin A would be resistant to most aminoglycoside-inactivating enzymes (4, 9). These data also show that fortimicin A probably will not act on permeability mutants.

MIC tests should be controlled by including reference strains in the system whenever these tests are performed (11). The strains listed in Table 6 are ones that have been used extensively in several laboratories. The range and mode MICs that we obtained in this study for fortimicin are shown in Table 6. For tests with aminoglycosides and *P. aeruginosa*, it is particularly important to include *P. aeruginosa* ATCC 27853 to control the cation content in the medium. We recommend that all lots of Mueller-Hinton broth be performance tested for quality control purposes and that the values in Table 6 be used as tentative standards if the broth is to be used for antimicrobial susceptibility tests.

In conclusion, fortimicin A has a wide spectrum of antibacterial activity, including activity against most of the clinically important aerobic

TABLE 6. *MICs of fortimicin A for five reference strains obtained in multiple tests*

Reference strain	ATCC no.[a]	Fortimicin MICs (range, μg/ml)	
		Cat (+)[b]	Cat (−)[b]
S. aureus	25923	0.5–1 (1)[c]	0.5–1 (0.5, 1)[d]
S. aureus	29213	1–2 (1)	0.5–2 (1)
S. faecalis	29212	16–64 (64)	16–64 (32)
E. coli	25922	2–4 (2, 4)[d]	0.5–4 (2)
P. aeruginosa	27853	16–64 (32)	2–8 (4)

[a] American Type Culture Collection number.

[b] Cat (+), Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter); Cat (−), unsupplemented Mueller-Hinton broth.

[c] Mode MIC is given in parentheses.

[d] The true MIC may be halfway between these two values, but when many tests are performed, both values occur with equal frequency.

and facultatively anaerobic bacteria, except *P. aeruginosa*, *P. cepacia*, *P. maltophilia*, *P. acidovorans*, and streptococci. It is equally active against methicillin-susceptible and -resistant *S. aureus* and β-lactamase-negative and -positive *N. gonorrhoeae* and *H. influenzae*. The drug is bactericidal, and its action on *P. aeruginosa* is affected by cations. MICs are substantially affected when the inoculum is raised from 10^5 to 10^7 CFU/ml. Fortimicin A is unaffected by most aminoglycoside-inactivating enzymes.

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