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Fortimicin A: Collaborative In Vitro Susceptibility Comparison with Amikacin and Gentamicin Against 11,840 Clinical Bacterial Isolates

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The susceptibility of 11,840 clinical bacterial isolates to fortimicin A was determined by agar dilution or broth microdilution methods and compared with their susceptibility to amikacin and gentamicin. In general, the in vitro activity of fortimicin A was essentially the same as that of amikacin. Significant exceptions were the increased effectiveness of fortimicin A against *Serratia marcescens* and the greater activity of amikacin against *Pseudomonas* and other nonfermentative gram-negative bacilli. On a weight-for-weight basis, gentamicin showed greater activity than the other two antimicrobial drugs against most species; *S. marcescens* was the major exception. However, at concentrations equivalent to achievable nontoxic serum levels, the proportion of isolates inhibited by the three drugs was quite comparable. There were several strains with unusually high resistance to one or more of the tested antibiotics. These usually occurred in one of the six participating institutions and could be traced to specific enzyme-producing or permeability mutants endemic to that particular institution.

Fortamine cyclitol-containing antimicrobials were obtained from *Micromonospora olivoasterospora* (2, 10, 11). The most promising compound, fortimicin A (XK-70-1), is a novel pseudodisaccharide antibiotic with *chiro* cyclitol stereochemistry (Fig. 1). This new aminoglycoside possesses antimicrobial activity similar to that of amikacin and kanamycin and markedly superior to that of the related derivative, fortimicin B (5, 10).

In this study we compared the in vitro antimicrobial activity of fortimicin A with that of amikacin and gentamicin C complex. A total of 11,840 current clinical isolates were tested at six microbiology laboratories in five widely separated geographic regions.

MATERIALS AND METHODS

Antibiotics. Gentamicin C complex sulfate was obtained from Schering Corp., Bloomfield, N.J. The amikacin sulfate standard powder was kindly supplied by Bristol Laboratories, Syracuse, N.Y. Fortimicin A sulfate was provided by Abbott Laboratories, North Chicago, Ill.

Bacterial isolates. The organisms employed in this study were consecutive clinical strains isolated in the clinical microbiology laboratories of Kaiser Foundation Hospitals and St. Vincent Hospital (Portland, Ore.), Sacramento Medical Center (Sacramento, Calif.), The Cleveland Clinic Foundation (Cleveland, Ohio), St. Francis Hospital (Wichita, Kans.), and Northwestern Hospitals (Chicago, Ill.). A total of 11,840 aerobic and facultative anaerobic organisms were tested. Each isolate was processed and identified by standardized procedures as previously described (4, 6-8).

Antimicrobial susceptibility testing. Minimimum inhibitory concentrations (MICs) of the three aminoglycosides were determined by either agar dilution or broth microdilution techniques. For microdilution tests, Mueller-Hinton broth (Difco) supplemented with 50 mg of calcium per liter and 25 mg of magnesium per liter was dispensed in plastic trays by using an MIC-2000 (Cooke Laboratory Products, Alexandria, Va.) as previously described (4, 6-8).

The agar dilution method was performed as described by Ericsson and Sherris (3), utilizing Mueller-Hinton agar and an inoculum replicating device of Steers et al. (13). Media, inoculating methods, incubation, and interpretation were rigidly controlled using standard performance characteristics, e.g., expected modal MIC on quality control organisms. Each spot contained approximately 5×10^4 colony-forming units. The plates were incubated at 35°C for 15 to 18 h.

MICs were defined as the lowest antimicrobial concentration totally inhibiting bacterial growth (no growth, a faint haze at inoculum site, or no more than one colony).

Media for testing *Streptococcus pneumoniae* and several beta-hemolytic streptococci were supplemented; Mueller-Hinton broth was used with 5% peptic digest of horse cells or Mueller-Hinton agar with 5% sheep erythrocytes.

Quality control organisms for which the MICs were known were tested daily in parallel with the unknown clinical strains. These quality control organisms included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 or 29213, *Streptococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853. Acceptable and comparable results were obtained in all collaborating laboratories. Less than 1% of all quality control MICs were outside of the ±1 dilution limits, a finding similar to that in earlier collaborative efforts (4, 6–8).

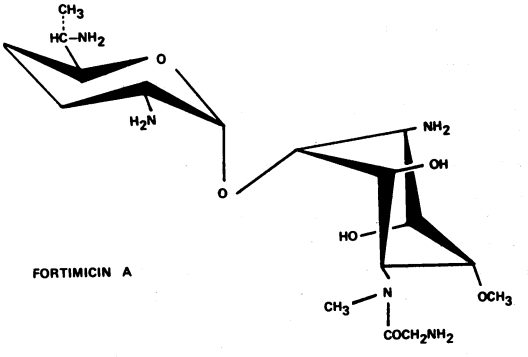

FIG. 1. *Structural characteristics of fortimicin A.*

For statistical analysis of the differences in susceptibility comparing the three aminoglycosides or six institutions, the Kalmogorov-Smironov two-sample (points on cumulative percentage curve) test of significance was employed (9).

RESULTS

The modal MICs and those required to inhibit 75 and 90% of the enteric isolates are shown in Table 1. Two dilution susceptibility methods were used; all modal results by species were within 1 $\log_2$ dilution interval. The antimicrobial activities of fortimicin A and amikacin were very similar against the *Enterobacteriaceae* when modal and median (not shown) results were compared. By MIC 90% endpoints, amikacin was generally twofold more active than fortimicin A against most species. Nearly equal inhibitory effects were found with *E. coli*, *Citrobacter diversus*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, morganella, and *Providencia stuartii*. On a weight-to-weight basis, gentamicin was generally superior to either fortimicin A or amikacin against the *Enterobacteriaceae*. Only against *Serratia marcescens*, *C. diversus*, and *P. stuartii* were amikacin or fortimicin A comparable or superior to gentamicin. Fortimicin A was significantly ($P < 0.001$) more effective against *S. marcesscens*.

TABLE 1. *Enterobacteriaceae*[a] *MICs of fortimicin A, amikacin, and gentamicin*

Organism (no. of isolates)	Modal MIC (μg/ml)			MIC 75 (μg/ml)			MIC 90 (μg/ml)		
	Fort	Amik	Gent	Fort	Amik	Gent	Fort	Amik	Gent
Citrobacter diversus (127)	≤0.5	≤0.5	≤0.5	2	2	2	2	2	4
C. freundii (130)	≤0.5	≤0.5	≤0.5	2	2	≤0.5	4	2	≤0.5
Escherichia coli (3,759)	2	2	2	2	2	2	4	4	2
Enterobacter aerogenes (352)	2	2	≤0.5	2	2	≤0.5	4	2	≤0.5
E. agglomerans (83)	4	4	2	4	4	2	8	8	4
E. cloacae (406)	2	2	≤0.5	2	2	≤0.5	4	2	2
Klebsiella oxytoca (199)	2	2	≤0.5	2	2	≤0.5	2	2	2
K. pneumoniae (1,010)	2	2	≤0.5	2	2	≤0.5	4	2	2
Morganella morganii (159)	2	2	≤0.5	4	2	≤0.5	4	4	2
Proteus mirabilis (791)	2	2	≤0.5	4	2	≤0.5	8	4	2
P. vulgaris (99)	2	2	≤0.5	2	2	2	4	2	2
Providencia rettgeri (58)	2	2	≤0.5	2	2	≤0.5	4	2	2
P. stuartii (35)	2	≤0.5	2	2	2	2	4	4	8
Salmonella enteritidis (95)	2	2	2	2	2	2	4	2	4
Serratia marcescens (438)	2	2	≤0.5	2	4	2	4	16	128[b]
Other *Enterobacteriaceae* species tested[c] (47)	2	2	≤0.5	4	2	≤0.5	4	2	2

[a] MIC 75 and MIC 90 values were calculated to closest $\log_2$ dilution step. *Enterobacteriaceae* were identified to species level based on taxonomic and nomenclature proposals of the Center for Disease Control (1). MIC 75 and 90, MICs inhibiting 75 and 90%, respectively, of the test organism. Fort, Fortimicin A; Amik, amikacin; Gent, gentamicin.

[b] Resistant MIC were skewed by endemic hospital organism populations (see Table 4).

[c] Includes *Shigella* sp. (8), *Klebsiella ozaenae* (1), *Serratia rubidea* (1), *Serratia liquefaciens* (2), *Providencia alcalifaciens* (1), *Hafniae alvei* (12), *E. coli* A-D group (20), and *Citrobacter amalonitica* (2).

Fortimicin A and amikacin possess nearly identical activity against most species of staphylococci and streptococci (Table 2). Gentamicin was fourfold more active than either fortimicin A or amikacin against the staphylococci. Amikacin inhibited more *S. aureus* (99%) at 16 μg/ml than gentamicin (96%) at 4 μg/ml or fortimicin A (96%) at 16 μg/ml. This was due to endemic fortimicin A- and gentamicin-resistant strains found in two participating institutions (see Table 4). Fortimicin A was the least active aminoglycoside against *Staphylococcus epidermidis* with only 83% of isolates inhibited by 16 μg/ml. Gentamicin also appeared more active when tested against most streptococcal species. All three drugs had high MICs for *S. faecalis*, though fortimicin A was fourfold more active (mode 32 μg/ml) than amikacin.

Table 3 tabulates the cumulative percentage susceptibility results of fortimicin A, amikacin, and gentamicin for the non-*Enterobacteriaceae* gram-negative bacilli. Fortimicin A modal MIC for *P. aeruginosa* was 32 μg/ml. In some hospitals resistant populations of endemic strains markedly affected the fortimicin A, gentamicin, and amikacin data (see Table 4). The percentage of *P. aeruginosa* strains inhibited by 16 μg of fortimicin A per ml (a clinically achievable level) ranged from 16 to 74% in the six participating hospitals. These data for pseudomonas were similar to those reported with kanamycin and were four- and eightfold less active than amikacin and gentamicin, respectively. Gentamicin had marked variation in activity, depending on the reporting hospital. Gentamicin susceptibility at ≤4 μg/ml ranged from a low of 62% to a high of 90%. The hospitals having the highest and lowest MIC modes used identical testing methods. The quality control strain values in each case were identical to those of the other hospitals.

Among the other nonenteric organisms, no statistical advantage could be detected favoring any one of the three aminoglycosides tested. None of the antimicrobials was effective against most *Pseudomonas* species. *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas putrifaciens*, and *Pseudomonas stutzeri* strains were usually susceptible to the tested compounds. Of the 30 other gram-negative nonenteric species, only *Alcaligenes* sp. and the flavobacteria were highly resistant.

TABLE 2. *Antimicrobial activity of fortimicin A, amikacin, and gentamicin tested on 2,879 isolates of the genera Streptococcus and Staphylococcus*

Organism (no. of isolates)	Antibiotic tested[a]	Cumulative % inhibited at MIC (μg/ml) of:						
		<0.5	2.0	4.0	8.0	16	32	128
Staphylococcus aureus (1, 349)	Fort	44	92[b]	95	95	96	96	99
	Amik	48	95	98	99	99	99	100
	Gent	89	95	96	97	99	99	100
Staphylococcus epidermidis (496)	Fort	73	79	80	81	83	89	97
	Amik	58	86	96	99	99	99	99
	Gent	78	83	87	94	98	99	100
Streptococcus agalactiae (31)	Fort	3	6	10	16	32	61	100
	Amik	3	6	10		16	32	97
	Gent	6	19	23	48	71	94	100
Streptococcus faecalis (897)	Fort		1	1	5	19	61	98
	Amik		1	2	3	5	13	51
	Gent	1	7	17	35	76	95	99
Streptococcus group D, not *faecalis*[c] (50)	Fort		2	10	30	60	94	100
	Amik	6		14	24	44	70	96
	Gent	10	36	58	82	94	100	
Streptococcus pneumoniae (13)	Fort	63	69		77	100		
	Amik	46	63	69			85	100
	Gent	54	63	69	92		100	
Streptococcus pyogenes (7)	Fort	14	57	86	100			
	Amik	14	28	86		100		
	Gent	57	100					
Streptococcus viridans group (36)	Fort	50	53	64	75	92	97	100
	Amik	22	58		71	84	89	100
	Gent	45	66	87	89	100		

[a] Fort, Fortimicin A; Amik, Amikacin; Gent, gentamicin.
[b] Underlined percentage is modal MIC.
[c] Includes *Streptococcus bovis* (27), *S. durans* (6), and *S. faecium* (17).

Fortimicin A MICs against *E. coli* and *P. aeruginosa* are shown in Fig. 2A and B. Marked statistical differences ($P < 0.001$) were found in the fortimicin A susceptibility between various hospitals. In some instances, an institution may have had the most susceptible or resistant organism population, depending on species. In all cases, no significant variation in quality control

TABLE 3. *Antimicrobial activity of fortimicin A, amikacin, and gentamicin tested on 1,173 isolates of non-Enterobacteriaceae gram-negative organisms*

Organism (no. of isolates)	Antibiotic tested[a]	Cumulative % inhibited at MIC (μg/ml) of:						
		<0.5	2.0	4.0	8.0	16	32	128
Acinetobacter calcoaceticus subsp. *anitratus* (123)	Fort	3	44[b]	72	80	85	88	93
	Amik	19	68	86	91	93	98	98
	Gent	41	84	87	91	94	97	100
subsp. *lwoffii* (13)	Fort		85	92	100			
	Amik	23	100					
	Gent	85	92	92	92	92	100	
Pseudomonas aeruginosa (881)	Fort	1	3	7	14	33	60	84
	Amik	6	24	57	78	88	94	99
	Gent	9	33	74	86	93	95	97
Pseudomonas spp.[c] (126)	Fort	2	14	16	17	35	44	67
	Amik	10	29	37	50	56	63	93
	Gent	17	31	47	58	72	87	95
Other gram-negative bacilli[d] (30)	Fort	13	37	40	43	50	60	67
	Amik	13	37	43	60		63	77
	Gent	33	47	57	60	67	70	77

[a] Fort, Fortimicin A; Amik, amikacin; Gent, gentamicin.
[b] Underlined percentage is modal MIC.
[c] Includes *Pseudomonas alcaligenes* (1), *P. cepacia* (2), *P. fluorescens* (4), *P. maltophilia* (37), *P. putida* (3), *P. putrifaciens* (1), *P. stutzeri* (1), and *Pseudomonas* sp. NOS (77).
[d] Includes *Aeromonas hydrophilia* (2), *Alcaligenes* sp. (7), *Flavobacterium* sp. (9), *Moraxella* sp. (11), and *Pasteurella multocida* (1).

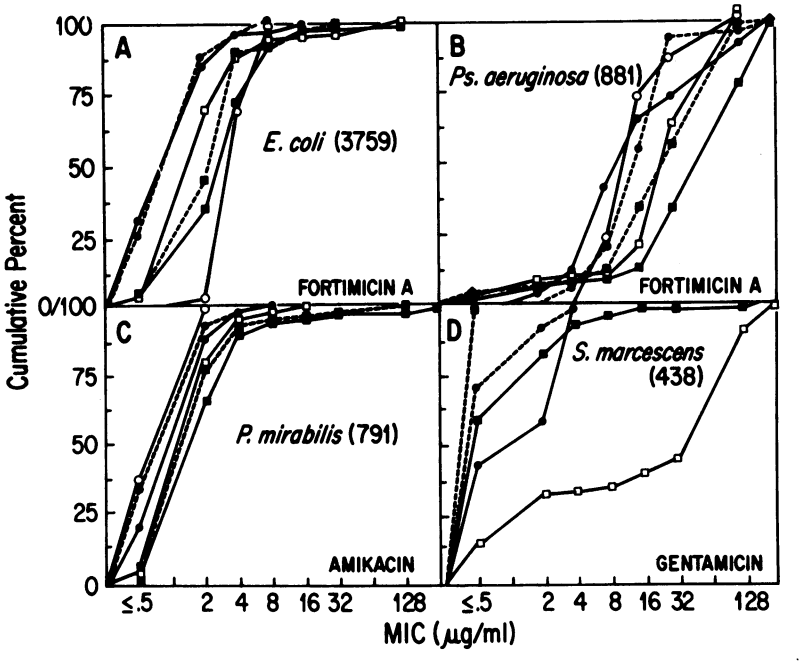

FIG. 2. *Cumulative percentage curves for the susceptibility of four bacterial species against amikacin, fortimicin A, and gentamicin, comparing Cleveland Clinic (■—■), Kaiser Foundation Hospitals (■- - -■), Sacramento Medical Center (□—□), Northwestern Memorial Hospital (●—●), St. Francis Hospital (●- - -●), and St. Vincent Hospital (○—○).*

organism modal MICs was detected. In Fig. 2C the amikacin MICs are shown for *Proteus mirabilis*. Here, no statistically valid ($P > 0.05$) differences were noted.

Figure 2D demonstrates the profound variations often found in gentamicin susceptibility. Among the study hospitals, the *S. marcescens* susceptibility pattern ranged from a modal MIC of ≤0.5 to one of 128 μg/ml. When differences were found among the endemic gram-positive or enteric organism populations in the hospitals, the most common pattern showed inhibition by all three compounds at various levels within the clinically susceptible range. In addition, variations between fortimicin A and amikacin were less likely than between fortimicin A and gentamicin. Less frequently encountered were single gentamicin resistance or combined resistance to fortimicin A, amikacin, and gentamicin.

Among the nonenteric gram-negative bacilli, the MICs of fortimicin A generally varied proportionately with gentamicin MICs. The fortimicin A marginal inhibitory effect (all-laboratory mode of 16 to 32 μg/ml) on pseudomonas organisms was partially negated by those resistance mechanisms found in two collaborating hospitals (see Fig. 2B). Similarly, an *Acinetobacter calcoaceticus* subsp. *anitratus* strain demonstrated marked resistance to fortimicin A, a high modal gentamicin MIC, and susceptibility to amikacin.

Five bacterial species (eight organisms) that harbored aminoglycoside resistance were found in high frequency (greater than 10% of total species isolated) in these facilities (Table 4). Several patterns were identified, ranging from total high-level aminoglycoside resistance to that of isolated tobramycin inactivity at an institution principally using that aminoglycoside. Representative type strains were processed to determine mechanisms of resistance by G. Miller and A. Waitz of Schering Laboratories. The results of these studies are also shown in Table 4.

DISCUSSION

Fortimicin A and B are novel new disaccharide aminoglycosides produced by *M. olivoasterospora* (2, 5, 10, 11). The structural qualities of fortimicin A protect it from most commonly encountered aminoglycoside-inactivating enzymes (10, 12). Fortimicin A has significantly increased antimicrobial activity as compared to fortimicin B (10), but lacks the pseudomonas antimicrobial features common to amikacin, gentamicin, and tobramycin (5, 10). Animal and human oto-renal toxicity has yet to be reported.

This study demonstrates that fortimicin A antimicrobial activity is comparable (modes and medians) to that of amikacin against staphylococci and the *Enterobacteriaceae* tested. The only exception was *S. marcescens*, for which the amikacin and gentamicin MIC 90s were 2- to 16-fold higher than that of fortimicin A. Both fortimicin A and amikacin were more active against *P. stuartii* than gentamicin, whose 8 μg/ml mode was within the resistant range. Overall, 90% or more of the *Enterobacteriaceae* were inhibited by 4, 4, and 2 μg of fortimicin A, amikacin, and gentamicin per ml, respectively. In most instances, amikacin and fortimicin A were effective against gentamicin-resistant organism subpopulations.

Mueller-Hinton agar and broth media used in this study contained magnesium and calcium

TABLE 4. *Five frequently encountered (>10%) endemic microbial species with aminoglycoside resistance found in study hospitals*

Organism (type strain)	MIC (μg/ml)					Resistance mechanism[a]
	Fortimicin A	Amikacin	Gentamicin	Kanamycin	Tobramycin	
Acinetobacter calcoaceticus subsp. *anitratus* (C5)	>64	16	16	>64	4.0	Unknown
Pseudomonas aeruginosa (15542)	>64	>64	64	>64	64	Permeability (high level)
Pseudomonas aeruginosa (22070)	>64	32	16	>64	4.0	Permeability (low level)
Serratia marcescens (G91)	4.0	2.0	>64	>64	>64	ANT(2″) + AAC(6′)
Staphylococcus aureus (SNT909)	4.0	2.0	≤0.125	>64	8.0	ANT(4′)
Staphylococcus aureus (SF139)	>64	4.0	>64	>64	>64	APH(2″) + AAC(6′)
Staphylococcus epidermidis (W181)	>64	4.0	8.0	>64	>64	APH(2″) + AAC(6′)

[a] Resistance mechanisms were determined by G. Miller and A. Waitz, Schering Corps., Bloomfield, N.J. ANT(2″), 2″-*O*-Adenylylation; AAC(6′), 6′-*N*-acetylation; ANT(4′), 4′-*O*-adenylylation; APH(2″), 2″-*O*-phosphorylation.

cations approximating in vivo free physiological concentrations (6). These additions greatly influence the *P. aeruginosa* susceptibility test results. However, the fortimicin A *P. aeruginosa* modal MIC compared favorably with prior reports using two different agar dilution methods (5, 9). Amikacin remains significantly ($P < 0.001$) more active against *P. aeruginosa* than gentamicin at clinically achievable concentrations in the study hospitals (6). Like other aminoglycosides, fortimicin A was relatively inactive against the frequently encountered streptococcus species. A total of 93% of the staphylococcal isolates were inhibited by 16 μg of fortimicin A per ml.

Several enzyme-producing and permeability mutant endemic hospital strains were identified during the study protocol. Some of these enzyme mechanisms have changed from those found in a study done less than 12 months before (6). These data emphasize the frequency of developing aminoglycoside resistance and the need for antimicrobial development combining resistance to inactivating enzymes and reduced antimicrobial-related toxicity.

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