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Comparison of Aminoglycoside Resistance Patterns in Japan, Formosa, and Korea, Chile, and the United States

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The resistance mechanisms of more than 2,000 aminoglycoside-resistant gram-negative aerobic bacteria were estimated by a method that assigned a biochemical mechanism based on susceptibility to selected aminoglycosides. Strains from hospitals in Japan, Formosa, and Korea (the Far East) were compared with strains from Chile and the United States. Of the strains from Chile, 90% had an aminoglycoside resistance pattern indicative of the 3-*N*-acetyltransferase [AAC(3)-V] enzyme. Of the strains from the Far East, 78% had susceptibility patterns suggesting the presence of AAC(6′) enzymes. In contrast, strains from the United States had a wider variety of resistance mechanisms including 2″-*O*-adenylyl-tidyltransferase [ANT(2″)], AAC(3), AAC(6′), and AAC(2′). Reflecting these differences in resistance patterns, the frequencies of resistance to gentamicin, tobramycin, dibekacin, and amikacin in strains from the United States were different from those in strains from the Far East. These differences seem to be correlated with different aminoglycoside usage in the two regions. In the United States, where gentamicin was the most widely used aminoglycoside, 92% of the strains were resistant to gentamicin, 81% were resistant to dibekacin, and 8.8% were resistant to amikacin. In the Far East, dibekacin and kanamycin were widely used in the past and more recently amikacin has been frequently used. Of the strains from this region, 99% were resistant to dibekacin, 85% were resistant to gentamicin, and 35% were resistant to amikacin.

Many factors, including the genetic characteristics of the resistance genes and plasmids, influence the spread of aminoglycoside resistance. In the past few years, several genes coding for aminoglycoside-modifying enzymes have been shown to occur on transposons (1, 10, 25), which aid their dissemination in hospitals and clinics. Phillips (21) and Daschner et al. (5) have noted that antibiotic usage is also an important factor in the spread of antibiotic resistance by providing selective pressure for the resistance gene products. Although most reports on the frequency of different types of aminoglycoside resistance are based on surveys of single hospitals, a few have compared resistance mechanisms in larger areas (13, 22). We were interested in how differences in antibiotic usage patterns would affect resistance mechanisms on a regional basis.

In the past few years strains collected throughout the world have been assigned an aminoglycoside resistance pattern (AGRP) on the basis of a susceptibility method (17). Most of these strains were obtained from three geographical regions (i) Japan, Formosa, and Korea; (ii) Chile; and (iii) the United States. In this study a comparison of the frequencies at which particular AGRPs were encountered in these three regions is given (K. Shimizu, T. Kumuda, W. Hseih, H.-Y. Chung, Y. Chong, R. Hare, G. H. Miller, F. Sabatelli, and J. Howard, Abstr. 13th Int. Congr. Chemother., (SE2.6/2, part, 52, p. 32–35, 1983). In two regions, the types of AGRPs seem to be correlated with aminoglycoside usage of the region. The AGRPs of the third region suggest that the pattern of aminoglycoside resistance in this smaller area (Chile) is like that of a hospital epidemic.

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MATERIALS AND METHODS

Strains. A total of 866 gram-negative strains resistant to one or more of the aminoglycosides gentamicin (Schering Corp.), tobramycin (Eli Lilly & Co., Indianapolis, Ind.), dibekacin (Meiji Seika), netilmicin (Schering, Corp.), and amikacin (Bristol Laboratories, Syracuse, N.Y.) were collected between 1980 and 1982 from 26 hospitals in a region comprising Japan (644), Formosa (74), and Korea (148). Similar strains (331) were obtained between 1980 and 1983 from 10 hospitals in Chile. Seven of these hospitals were in the capital city, Santiago, two were in northern Chile, and one was in Concepción. A total of 1,187 strains were obtained from 170 hospitals in the United States from 1974 through 1983, 50% of them being collected from 1978 through 1980. These strains were collected from hospitals having aminoglycoside resistance problems. Only unsolicited strains sent to our laboratories for determination of AGRPs from these three regions were included in the study. Duplicates of the strains originating in Chile and the Far East were specifically excluded. Patient and source identification of the U.S. strains suggest that few, if any, of these strains were from the same patient.

The strains were selected primarily on the basis of disk susceptibility. Specific cutoffs and methods varied with the country in which the strains were initially isolated. For this reason, all the strains were re-identified with the API 2OE system (Analytab Products, Plainview, N.Y.) and antibiotic susceptibilities were determined by using the National Committee for Clinical Laboratory Standards cutoffs for gentamicin, tobramycin, amikacin, and netilmicin (18). For comparative purposes, 1,580 strains susceptible to gentamicin, tobramycin, netilmicin, and amikacin were collected

TABLE 1. Activity of selected aminoglycosides against normal strains

Organism (no.)[a]	Geometric mean MICs (μg/ml) of:[b]										
	GM	TOB	SISO	DKB	NET	AMK	2'-NET	6'-NET	ASTM	Sch 21420	Sch 22591
Providencia spp. and *Proteus vulgaris* (30)	2.1	2.3	2.3	3.7	2.0	4.1	2.8	3.9	6.2	2.5	0.54
Serratia spp. (29)	0.68	1.5	0.59	2.2	1.1	2.7	4.3	1.5	2.7	1.5	0.42
Pseudomonas spp. (702)	0.58	0.31	0.35	0.43	0.90	1.4	4.8	4.9	7.7	1.6	0.33
Other gram-negative strains (637)[c]	0.93	0.97	0.76	1.0	0.8	3.4	2.6	3.0	3.8	2.0	0.69
Citrobacter spp. (32)	0.80	0.79	0.58	1.2	0.62	2.4	2.5	3.6	5.7	1.5	0.64
Enterobacter spp. (53)	0.5	0.59	0.42	0.81	0.41	2.2	2.5	4.1	2.7	1.1	0.30
E. coli (285)	1.2	1.3	1.0	1.2	0.97	4.2	1.9	1.7	3.8	2.2	0.95
Klebsiella spp. (161)	0.57	0.57	0.46	0.63	0.53	2.0	3.0	4.2	2.7	0.99	0.49
Proteus mirabilis (87)	1.5	1.3	1.3	1.8	1.6	6.3	6.5	6.2	7.2	7.0	1.6

[a] Number of strains tested.
[b] GM, Gentamicin; TOB, tobramycin; SISO, sisomicin; DKB, dibekacin; NET, netilmicin; AMK, amikacin; 2'-NET, 2'-*N*-ethyl-netilmicin; 6'-NET, 6'-*N*-ethyl-netilmicin; ASTM, astromicin, Sch 21420, HAPA-gentamicin B; Sch 22591, 5-episisomicin.
[c] Those listed in table plus a few (i.e., 19) strains of *Morganella*, *Salmonella*, and *Shigella*.

from the same sources at the same time and tested in a similar manner.

Antibiotic susceptibility. MICs of selected aminoglycosides were determined for each strain by macrobroth (17), agar (16), or microbroth dilution methods with unsupplemented Mueller-Hinton media (Difco Laboratories, Detroit, Mich.). The use of cation-supplemented media was found to be unsuitable for determining resistance profiles in *Pseudomonas* strains (*P. aeruginosa*, *P. fluorescens*, and *P. putida*), since the elevated MICs observed mask differences in relative potencies (14). For the microbroth MIC determinations, 100 μl of appropriately diluted aminoglycoside-containing broth was dispensed into a 96-well microtiter plate by using the Sandy Spring Quick Spense II (Sandy Spring Instrument Co., Ijamsville, Md.). The plates were stored at −20°C until use. The plates were slowly thawed at 4°C before inoculation with 5 μl of 1:100 dilutions of 24-h cultures. The MIC, the lowest drug concentration at which no visible growth was observed, was determined after 24 and 48 h. In addition to gentamicin, tobramycin, amikacin, netilmicin, and dibekacin, aminoglycosides tested included sisomicin (Schering Corp.), 2'-*N*-ethyl-netilmicin (Schering Corp.), 6'-*N*-ethyl-netilmicin (Schering Corp.), astromicin (fortimicin; Kyowa Hakko), HAPA-gentamicin B (Sch 21420; Schering Corp. [15]), 5-episisomicin (Sch 22591; Schering Corp. [24]), and apramycin (Eli Lilly & Co.).

MIC susceptibility data were verified by disk susceptibility tests performed according to the National Committee for Clinical Labroatory Standards procedures (18). In addition, other classes of antibiotics were tested by these methods to determine the total resistance profile of each strain.

RESULTS

AGRPs. The AGRPs were determined for all strains. The aminoglycosides tested were selected so that unique resistance patterns would be obtained for the known aminoglycoside resistance mechanisms (17). The aminoglycosides chosen did not allow the classification of the phosphotransferases [APH(3')] causing resistance to kanamycin or neomycin or those enzymes, 3''-*O*-nucleotidyltransferase [ANT(3'')], 3-phosphotransferase [APH(3'')], and 6-phosphotransferase [APH(6)], producing resistance to streptomycin or spectinomycin. Therefore, strains classified as normal were susceptible to all the tested aminoglycosides but may have contained the above phospho- and adenylyltransferases. In addition, these enzymes undoubtedly occurred in combination with the other enzymes studied.

The concentration of the selected aminoglycosides needed to inhibit growth of normal (susceptible) strains are shown in Table 1. Gentamicin, tobramycin, netilmicin, dibekacin, Sch 22591, and sisomicin had similar potencies against most strains. Differences (two- to threefold) occurred with *Pseudomonas* and *Serratia* strains. Amikacin, Sch 21420, astromicin, and the netilmicin derivatives 2'- and 6'-*N*-ethyl-netilmicin were fourfold less potent than were the above-mentioned group against most gram-negative strains. However, against *Providencia* spp. and *Proteus vulgaris* strains they were only twofold less potent. Changes from these relative potencies as well as the base-line mean MICs shown in Table 1 were very important in determining whether other strains were susceptible or resistant to the various aminoglycosides.

The susceptibility pattern for each resistant strain was correlated with those of strains containing biochemically defined aminoglycoside-modifying enzymes. These strains were of two types. The first group of 32 gram-negative and 10 gram-positive isolates were ones for which biochemical data have been published. They included ANT(2'') (two *Escherichia coli*, six *Klebsiella*, one *Pseudomonas*, one *Salmonella*, and one *Serratia* strain), ANT(2'') + AAC(6') (two *Serratia* strains), AAC(2') (one *Providencia* strain), AAC(6')-IV (one *E. coli*, one *Moraxella*, one *Pseudomonas* strain), AAC(6')-III (one *Pseudomonas* strain), AAC(3)-I (two *Acinetobacter* strains), one *E. coli*, two *Klebsiella*, and three *Pseudomonas* strains), AAC(3)-IV (one *E. coli* and one *Salmonella* strain), AAC(3)-V (two *Klebsiella* and two *Serratia* strains), ANT(4') (four *Staphylococcus* strains), APH(2'') + AAC(6') (five *Staphylococcus* strains), and APH(3)-IV (one *Staphylococcus* strain). This group of strains consisted of no more than one isolate of a specific genus from a given source. The second group of standard strains was much larger because it included multiple isolates from a given source. The results of susceptibility tests with these strains were used to define the AGRPs listed in Table 2. The results of the susceptibility tests with the experimental strains are shown in Table 3, grouped according to the defined AGRPs. Subgroups of the various patterns are listed as a, b, and c to distinguish them from the established enzyme nomenclature. Subgroups were necessary since the

TABLE 2. Correlation among AGRP, susceptibility, and modifying enzymes

AGRP	Observed resistance patterns for[a,b]:											Corresponding enzymatic mechanisms (reference)
	GM	TOB	SISO	DKB	NET	AMK	2′-NET	6′-NET	ASTM	Sch 21420	Sch 22591	
AAC(3)-a	R		R						R			3-*N*-acetyltransferases; AAC(3)-I (7)
AAC(3)-b	R		R		R		R	R				3-*N*-acetyltransferases; unclassified
AAC(3)-c	R	R	R	R	R		R	R				3-*N*-acetyltransferases; AAC(3)-IV, -V (7, 8)
AAC(6′)-a		R	R	R	R/S[c]	R/S[c]	R			R/S[c]	R	6′-*N*-acetyltransferases; AAC(6′)-IV (12)
AAC(6′)-b	R	R	R	R	R		R				R	6′-*N*-acetyltransferases; AAC(6′)-III (12)
AAC(6′)-c							R					6′-*N*-acetyltransferases; AAC(6′)-I, -II or unclassified (12)
AAC(2′)-a								R				2′-*N*-acetyltransferases (low level)
AAC(2′)-b	R	R	R	R	R			R				2′-*N*-acetyltransferases (7)
ANT(2″)	R	R	R	R								2″-*O*-nucleotidyltransferases (7)
ANT(2″) + AAC(6′)	R/I[d]	R	R	R	R/S[c]	R/S[c]	R			R/S[c]	R	2″-*O*-nucleotidyltransferases + 6′-*N*-acetyltransferases
Permeability	R	R	R	R	R	R	R	R	R	R	R	Uptake mutants, electron transport mutants, etc. (2)

[a] See Table 1, footnote *b*.
[b] R, Resistant; S or blank, susceptible; I, intermediate.
[c] Resistance phenotype varied depending upon the level of AAC(6′) activity.
[d] Resistance phenotype varied depending upon the level of ANT (2″) activity.

AGRPs were not always homogeneous groupings of specific enzymes. Strains known to contain the AAC(3)-IV and the AAC(3)-V enzymes had the same basic AGRP: AAC(3)-c. The AGRPs AAC(3)-a and AAC(6′)-c also contain strains bearing different enzyme complements. Biochemical studies of the enzyme(s) causing the AAC(3)-b AGRP have not been published. The AAC(6′)-c and AAC(2′)-a AGRPs were essentially susceptible strains that were resistant to either 2′- or 6′-*N*-ethyl-netilmicin, which was considered to be an indication of low-level acetylation activity.

The two experimental netilmicin derivatives, 2′ and 6′-*N*-ethyl-netilmicin, were very important in distinguishing strains containing AAC(3)-c, AAC(6′)-b, or AAC(2′)-b, all of which were resistant to gentamicin, tobramycin, sisomicin, dibekacin, and netilmicin. Since 2′- and 6′-*N*-ethyl-netilmicin are known (Table 1) to have equal activity against normal strains, ≥ eightfold differences in relative activity were considered as evidence for either 2′- or 6′-acetylating activity. Other compounds were also used to confirm specific AGRPs. In gram-negative bacteria, resistance to astromicin is only caused by AAC(3)-I (20), and resistance to apramycin is only caused by AAC(3)-IV (6). About 60 strains in this study that had the AAC(3)-c AGRP were shown to be apramycin susceptible; they therefore presumably contain the AAC(3)-V enzyme (8).

Resistance patterns in *Pseudomonas* strains. *Pseudomonas* strains from 25 hospitals in the Far East, 2 hospitals in Chile, and 106 hospitals in the United States were tested. The AGRPs found in *Pseudomonas* strains are summarized in Table 4. *Pseudomonas* strains accounted for a high percentage (36.1%) of resistant strains from the United States but made up only 15 to 20% of the resistant strains collected from the two other regions. In Chile, most of the *Pseudomonas* strains produced one of the 3-*N*-acetyltransferases.

TABLE 3. Activity of selected aminoglycosides against resistant strains

AGRP	Geometric mean MICs[a] (μg/ml) of[b]:										
	GM	TOB	SISO	DKB	NET	AMK	2′-NET	6′-NET	ASTM	Sch 21420	Sch 22591
AAC(3)-a	45	0.44	20	ND[d]	1.6	1.1	6.5	8.5	299	1.5	0.26
AAC(3)-b	96	1.1	69	ND	42	1.0	141	152	ND	0.57	
AAC(3)-c	111	21	92	44	38	1.9	109	124	3.6	1.4	1.9
AAC(6′)-a	2.3	36	22	70	67	25	118	3.0	4.5	10	6.5
AAC(6′)-b	34	21	68	69	94	1.9	133	7.0	7.7	2.3	30
AAC(6′)-c	0.97	2.9	1.3	4.7	2.5	2.2	19	0.93	2.1	1.4	0.78
AAC(2′)-a	2.3	3.4	2.7	4.4	4.0	1.7	1.1	18	4.0	1.6	0.44
AAC(2′)-b	43	28	27	47	38	4.0	5.9	92	5.8	4.8	0.87
ANT(2″)	36	36	23	59	0.75	1.9	3.2	3.9	3.1	1.2	0.74
ANT(2″) + AAC(6′)	48	76	49	108	33	14	83	2.7	4.3	6.0	7.7
Permeability	14	10	9.9	18	19	22	48	52	36	30	6.4

[a] Includes strains from all three regions.
[b] See Table 1, footnote *b*.
[d] ND, Not determined.

TABLE 4. AGRPs in *Pseudomonas* strains

Parameter	Distribution among[a]: Japan, Formosa, Korea		Chile		United States	
Total no. of strains	175		47		429	
% resistant gram-negative strains	20.2%		14.2%		36.1%	
AGRP						
ANT(2″)	4.0%*		0%*		27.0%	
AAC(6′)-a	9.1%		0%		1.2%	
AAC(6′)-b	70.3%	88.0%	2.1%	2.1%	28.9%	31.3%
AAC(6′)-c	2.9%		0%		1.2%	
ANT(2″) + AAC(6′)	5.7%		0%		0%	
AAC(3)-a	0%		31.9%		8.9%	
AAC(3)-b	0%	1.1%	6.4%	93.6%	11.7%	26.2%
AAC(3)-c	1.1%		55.3%		5.6%	
Permeability	6.8*		4.3%*		15.6%*	

[a] All differences among regions were highly significant ($P = 0.0001$) by the chi-square test except those marked with an asterisk.

These strains were all resistant to gentamicin and suscpetible to amikacin; the major AGRP [AAC(3)-c] showed resistance to tobramycin, dibekacin, and netilmicin as well. As stated above, owing to the susceptibility to apramycin of selected AAC(3)-c strains from Chile, most of these strains appeared to contain the AAC(3)-V enzyme. The resistance profiles of *Pseudomonas* strains from the Far East indicated that most strains contained 6′-*N*-acetyltransferases, some of which [AAC(6′)-a] conveyed resistance to amikacin. The resistance profiles for *Pseudomonas* strains from the United States indicated a wider distribution of resistance mechanisms, with the strains as likely to contain a 2″-*O*-adenylyltransferase as they were to contain the 3- and 6′-*N*-acetyltransferases.

Some *Pseudomonas* strains (5 to 15%) from all regions were resistant to all the aminoglycosides tested. This type of resistance was considered to be most probably due to decreased uptake of aminoglycosides (9) and is indicated as permeability. In addition, many *Pseudomonas* strains which were classified as normal could have been considered to be resistant as a result of decreased permeability (4) on the basis of MIC tests in cation-supplemented media. An especially large number of this type of strain was collected in the United States. If these strains had been classified as permeability resistant instead of normal, the percentage of *Pseudomonas* strains within this AGRP would have increased to 30 to 40%.

TABLE 5. AGRPs in Serratia strains

Parameter	Distribution among[a]: Japan, Formosa, Korea		Chile	United States	
Total no. of strains	370		6	228	
% resistant gram-negative strains	42.7%		1.8%	19.2%	
AGRP					
ANT(2″)	2.2%		0%	18.4%	
AAC(6′)-a	21.1%		0%	12.0%	
AAC(6′)-c	5.4%	97.9%	0%	9.2%	69.0%
ANT(2″) + AAC(6′)	71.4%		0%	47.8%	
AAC(3)-a	0%		0%	1.3%	11.4%
AAC(3)-c	0%		100%	10.1%	
Permeability	0%*		0%	0.4*	

[a] All differences between the Far East and the United States were highly significant ($P = 0.0001$) by the chi-square test except those marked with an asterisk.

TABLE 6. AGRPs in *Providencia* and *Proteus vulgaris* strains

Parameter	Distribution among[a]: Japan, Formosa, Korea		Chile		United States	
Total no. of strains	49		15		117	
% resistant gram-negative strains	5.7%		4.5%		9.9%	
AGRP						
ANT (2″)	10.2%		0%		1.7%	
ANT (2″) + AAC (6′)	46.9%*		0%		0%	
AAC(3)-c	10.2%		80.0%*		10.2%	
AAC(2′)-a	4.1%	30.6%	13.3%	20.0%	10.2%	88.0%*
AAC(2′)-b	26.5%		6.7%		77.8%	
Permeability	2.0%		0%		0%	

[a] Differences among regions were *not* highly significant ($P > 0.0001$) by the chi-square test except those marked with an asterisk.

Resistance patterns in *Serratia* strains. A total of 604 *Serratia* strains were collected from 25 hospitals in the Far East, 54 hospitals in the United States, and only 1 in Chile. These strains occurred frequently among the aminoglycoside-resistant strains (Table 5). Although rarely observed in the strains from Chile, they were very prevalent in the strains from the Far East (42.7%) and intermediate in occurrence in the strains from the United States (19.2%). Among the Chilean strains, only the AAC(3)-c AGRP was observed. The resistance patterns of almost 98% of the *Serratia* strains from the Far East suggested the presence of 6′-*N*-acetyltransferases. Similarly, almost 70% of the *Serratia* strains from the United States showed 6′-acetylating activity; however, AAC(3) and ANT(2″) enzymes also occurred.

Resistance patterns in *Providencia* strains and *Proteus vulgaris*. Aminoglycoside-resistant *Providencia* and *Proteus vulgaris* strains (Table 6) were obtained from 41 hospitals in the United States. They generally (88%) had a resistance pattern associated with the AAC(2′) enzyme. However, 12 strains with the AAC(3) pattern were obtained from a small subgroup of three hospitals in the United States late in the study. Although the AAC(2′) pattern occurred in *P. vulgaris* and *Providencia* strains from the other regions, it was not as common. The predominant pattern in Chile, where 15 strains from five hospitals were studied, was AAC(3)-c. In the Far East, strains were collected from 20 hospitals and the ANT(2″) + AAC(6′) pattern was most common. Thus, the patterns seen in the isolates from Chile and the Far East were similar to those observed in *Serratia* strains.

Resistance patterns of "other" gram-negative bacteria. Within a given region, the AGRP patterns in *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Proteus mirabilis*, and *Salmonella* strains were very similar and therefore will be discussed as one group. AGRPs for a small

TABLE 7. AGRPs in other gram-negative strains[a]

Parameter	Distribution among[b]: Japan, Formosa, Korea		Chile		United States	
Total no. of strains	370		263		413	
% resistant gram-negative strains	42.7%		79.5%		34.8%	
AGRP						
ANT (2'')	46.0%		0%		82.1%	
AAC(6')-a	12.9%		0%		0.2%	
AAC(6')-b	0%	50.8%	0.4%	0.4%*	0.2%	2.6%*
AAC(6')-c	0.4%		0%		2.2%	
ANT(2'') + AAC(6')	37.5%		9%		0%	
AAC(3)-a	0%		0%		4.1%	
AAC(3)-c	2.2%	2.2%	96.6%	96.6%	7.7%	11.8%
AAC(2')-a	0%	0.4%*	0%	0%*	0.2%	0.2%*
AAC(2')-b	0.4%		0%		0%	
Permeability	0.7%*		3.0%*		3.1%*	

[a] Includes strains of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Proteus mirabilis*, and *Salmonella*.
[b] All differences among regions were highly significant ($P = 0.0001$) by the chi-square test except those marked with an asterisk.

number of *Acinetobacter* strains were also determined; however, these seemed to be different and were therefore not included. Strains were obtained from 22 hospitals in the Far East, 9 in Chile, and 62 in the United States. Similar regional differences in resistance patterns were observed for all the other gram-negative genera (Table 7). This group of strains accounted for a high percentage (79.5%) of the resistant strains encountered in Chile but a smaller percentage of those from the Far East and the United States. The AGRP AAC(3)-c was very common (96.6%) in strains from Chile. Most strains (82.1%) from the United States had a resistance pattern consistent with ANT(2''). Many strains (46.0%) from the Far East also had an ANT(2'') pattern. However, as with *Serratia*, *Providencia*, and *Proteus vulgaris* strains from this region, many (37.5%) had resistance patterns consistent with the combination of ANT(2'') and AAC(6') enzymes.

Resistance patterns of all gram-negative bacteria. The frequency of occurrence of resistance profiles for all tested strains is shown in Table 8. The ANT(2'') pattern which reflects resistance to gentamicin, tobramycin, dibekacin, and sisomicin was the most frequently occurring AGRP (645 strains) in this survey. It was especially common in *Klebsiella* (291 strains), but also occurred in *Escherichia* (73 strains), *Enterobacter* (52 strains), and *Citrobacter* (30 strains) and, as mentioned previously, was seen in both

TABLE 8. AGRPs in gram-negative strains

Parameter	Distribution among[a]: Japan, Formosa, Korea		Chile		United States		Resistance phenotype
Total no. of strains	866		331		1,187		
No. of hospitals sending isolates	26		10		170		
AGRP							
ANT(2'')	16.7%		0%		42.0%		G, D, K
AAC(6')-a	14.9%		0%		2.9%		D, K
AAC(6')-b	14.2%	78.2%	0.6%	0.6%	10.4%	25.5%	G, D, K
AAC(6')-c	3.0%		0%		2.3%		D,[c] K[c]
ANT(2'') + AAC(6')	46.1%		0%		9.9%		G, D, K
AAC(3)-a	0%		4.5%		4.9%		G
AAC(3)-b	0%	1.5%	0.9%	95.4%*	4.2%	16.8%	G
AAC(3)-c	1.5%		90.0%		7.7%		G, D,[d] K[d]
AAC(2')-a	0.2%	1.8%*	0.6%	0.9%*	1.1%	8.8	G, D
AAC(2')-b	1.6%		0.3%		7.7%		
Permeability	1.7%*		3.0%*		6.8%*		G, D, K

[a] All differences among regions were highly significant ($P = 0.0001$) by the chi-square test except those marked with an asterisk.
[b] Resistance phenotype symbols: G, gentamicin; D, dibekacin; K, kanamycin.
[c] Strains for which dibekacin and kanamycin, which were increased relative to other compounds, showed intermediate MICs.
[d] Strains for which dibekacin and kanamycin showed raised MICs, which were less than that of gentamicin.

Pseudomonas (117 strains) and *Serratia* (42 strains) from the United States.

In the United States, specific AGRPs were associated with certain genera. That is, AAC(2′) AGRPs were found in *Providencia* and *Proteus* strains; AAC(6′) AGRPs were found in *Serratia* strains and ANT(2″) were found in other gram-negative organisms; *Pseudomonas* strains had several AGRPs; and AAC(3) AGRPs were spread throughout all genera.

Although no single AGRP was dominant in the strains from the Far East, the AGRPs indicative of 6′-acetylating activity [AAC(6′)-a, b, and c and ANT(2″) + AAC(6′)] accounted for 78.2% of all resistance. Overall, a large number of strains had a pattern of resistance consistent with the presence of both ANT(2″) and AAC(6′) enzymes. The regional differences observed between strains from the Far East and the United States were also reflected in the combination AGRP, ANT(2″) + AAC(6′). This pattern occurred in 109 strains of *Serratia* in the United States. However, in the Far East it occurred not only in *Serratia* spp. (264 strains) but also in *Pseudomonas* spp. (10 strains), *Providencia* spp. and *Proteus vulgaris* strains (23 strains), and other gram-negative bacteria (139 strains). The heterogeneous nature of resistance in this AGRP was most probably due to variable contributions of the two enzymes. For this reason, the strains were split into four subgroups on the basis of levels of AAC(6′) and ANT(2″) activity. Strains resistant to both amikacin and netilmicin (MICs of $\geq$ 16 μg/ml) were classified as having high levels of AAC(6′). MICs of gentamicin of $\geq$ 32 μg/ml were used to indicate high levels of ANT(2″) activity. Over 70% (72 to 78%) of these strains from both regions had high-level adenylylating activity as reflected by gentamicin MICs. However, a majority (62%) of the strains from the Far East had high levels of the AAC(6′) enzyme, as reflected by amikacin and netilmicin MICs, compared with only 31% of these strains from the United States.

DISCUSSION

In this study, the enzymatic mechanism of aminoglycoside resistance for over 2,000 strains was estimated by correlating observed susceptibility patterns to those of standard strains with biochemically identified aminoglycoside-modifying enzymes. The method allows one to conveniently classify a large number of strains. However, it is based on a phenotypic parameter and therefore could be misleading. To check the system, biochemical studies were carried out on 94 additional *Pseudomonas* strains between 1979 and 1980 (G. A. Jacoby, personal communication). When enzymatic activity was detected in a strain (ca. 75% of the time), it had been properly classified by the susceptibility pattern 87% of the time. However, 74% of the *Pseudomonas* strains classified as permeability resistant were found to contain either acetylating or adenylylating activity. On the basis of these results, the number of aminoglycosides tested was increased; the additional compounds such as astromicin [AAC (3)-I] and apramycin [AAC(3)-IV] more clearly identified acetylating enzymes. However, it is clear that strains labeled permeability resistant may contain specific aminoglycoside-modifying enzymes in addition to broad general resistance to all aminoglycosides. In the last few years, more than 100 experimental strains were evaluated either biochemically or by hybridization studies (J. Davies, F. Tenover, and W. Piepersberg, personal communications). Of these strains, 90% were classified appropriately by the susceptibility pattern method. When there was disagreement between the two methods, it usually occurred in strains having multiple aminoglycoside-modifying enzymes, where one pattern was "hidden" within another. For example, an ANT(2″) pattern (Gm^r, Tob^r) probably would not be detected in a strain which also contained AAC(3)-c (Gm^r, Tob^r, Net^r, 2′-Net^r, 6′-Net^r). This same type of error could occur in biochemical studies as well, if a strain contained two different acetylating enzymes with similar substrate profiles. Therefore, we feel that the susceptibility pattern method can be used to make a reasonable estimation of aminoglycoside resistance mechanisms.

There are numerous reports of infections caused by aminoglycoside-resistant strains in a single hospital. In some of these reports, the resistance is of a single type and is limited to a single genus, such as the finding of the AAC(3)-I enzymes in strains of *Serratia marcescens* in England (3) and an AAC(6′) enzyme in *Enterobacter* spp. in South Carolina (11). In other hospitals a single resistance mechanism has spread to strains of several different genera. O'Brien et al. (19) noted the spread of the ANT (2″) through most gram-negative genera in a Boston hospital. Schaberg et al. (23) described the course of nosocomial infection at Vanderbilt which started with a Gm^r *Serratia* strain but then spread to other strains, including *Klebsiella*.

The high incidence of a single AGRP such as AAC(3)-c in Chile is characteristic of the outbreaks in single hospitals described above. In this case a country (but primarily the city of Santiago) seems to be behaving like a hospital in which a single organism or plasmid has caused an epidemic. Some additional studies have been done on the plasmid of an AAC(3)-c strain from Chile (1), and the gene was associated with an IS140 structure. This might explain why this resistance gene spread rapidly into all genera throughout the country.

The resistance profiles for the Far East and the United States were more complex (Table 8). In the United States, the most common AGRPs, ANT(2″), AAC(3), AAC(2′), AAC(6′)-b, and ANT(2″) + AAC(6′), all cause resistance to gentamicin as shown by the resistance phenotype. A gm^s AGRP, AAC(6′)-a, was infrequently observed (2.9%). It is probable that the widespread use of gentamicin in the United States at and before the time of strain collection was responsible by selective pressure for the predominance in the United States of susceptibility patterns including gentamicin resistance. Of the resistant strains from the United States, 92% were resistant to gentamicin, 85% were resistant to tobramycin, 81% were resistant to dibekacin, and only 8.8% were resistant to amikacin.

In Japan, the kanamycin family of aminoglycosides (kanamycin A and B, dibekacin, tobramycin, and amikacin) accounted for over 60% of the aminoglycoside unit sales between 1980 and 1983 (audited sales data). The commonly observed AGRPs were ANT(2″), AAC(6′)-a, AAC(6′)-b, and ANT(2″) + AAC(6′), all of which cause resistance to dibekacin. The AAC(3) family of enzymes is almost completely absent (1.5%) from the strains from the Far East. Since these enzymes inactivate gentamicin, sisomicin, and netilmicin more readily than the kanamycin antibiotics tobramycin, dibekacin, and amikacin, the absence of these enzymes also may be correlated with selective pressure. Similarly, the AAC(2′) enzyme, which occurs infrequently in the Far East, acetylates gentamicin, sisomicin, and netilmicin but only dibekacin and tobramycin in the kanamycin family. Of the resistant strains from this region, 99% were resistant to dibekacin, 96% were resistant to tobramycin, only 85% were resistant to gentamicin, and over 35% were resistant to amikacin. These values were all signifi-

cantly different (by the chi-square test) from the corresponding ones from the United States ($P = 0.0001$).

In summary, different aminoglycoside resistance profiles have emerged in the United States and the Far East. Reflecting these differences in resistance patterns, different frequencies of resistance to gentamicin, tobramycin, dibekacin, and amikacin have occurred in the United States from those in Japan, Formosa, and Korea. These differences are correlated with different aminoglycoside usage in the two regions. The high incidence of the AAC(3)-c AGRP in Chile is characteristic of a localized outbreak.

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