Aminoglycoside Resistance and Aminoglycoside Usage: Ten Years of Experience in One Hospital

DALE N. GERDING,1,2* TOM A. LARSON,3 RITA A. HUGHES,1 MARY WEILER,1 CAROL SHANHOLTZER,2 AND LANCE R. PETERSON1,2

Infectious Disease Section, Medical Service,1 Laboratory Service,2 and Pharmacy Service,3 Veterans Affairs Medical Center, and University of Minnesota Medical School, Minneapolis, Minnesota 55417

Received 5 November 1990/Accepted 17 April 1991

For 10 years the 700-bed Minneapolis Veterans Affairs Medical Center has conducted a policy of carefully controlled aminoglycoside usage and monitoring of resistance of over 25,000 aerobic and facultative gram-negative bacillary isolates to the aminoglycosides. On two occasions during the 1980s, our experience of introducing amikacin at a high level of usage was associated with a significant reduction in resistance to gentamicin and tobramycin among gram-negative bacilli. Rapid reintroduction of gentamicin usage in 1982 after the first amikacin period was associated with a significant and rapid increase in gentamicin and tobramycin resistance. However, in 1986, gentamicin was again reintroduced to this institution at an initially modest level, and the percentage of usage of gentamicin was gradually increased over a 15-month period without a significant change in resistance to gentamicin, tobramycin, or amikacin while maintaining an overall 66% gentamicin usage and 30% amikacin usage. Aminoglycoside usage (measured as patient days) rose steadily from under 2,000 patient days per quarter in 1980 and 1981 to over 3,000 days per quarter in 1985. Since 1985, usage has declined to under 2,500 patient days per quarter in 1990. This usage rise and fall occurred during a steadily declining daily patient census that was 590 in 1980 and 465 in 1989. A move to a new hospital building in June 1988 was associated with an additional significant decline in resistance to all aminoglycosides (P < 0.05), continuing a trend that was evident for the year preceding the move. Resistance to aminoglycoside antibiotics is now at the lowest level in 10 years at this institution, with only one gram-negative organism, Pseudomonas aeruginosa, that exhibits more than 5% resistance to gentamicin and no gram-negative species that are more than 5% resistant to amikacin and tobramycin.

Experience during the 1970s in the United States with aminoglycoside resistance among gram-negative bacilli suggested that gentamicin and tobramycin might lose their effectiveness as therapeutic agents because of the widespread development of resistance (4, 7, 13, 15). Many of these resistant organisms were shown to carry resistance or R plasmids that bore the genetic information for enzymatic resistance and that could be transferred from organism to organism (6, 10, 11, 18). An outbreak of this type was initially noted at our institution in 1975, first among Klebsiella species, then among Serratia species, and eventually among multiple other members of the family Enterobacteriaceae (4, 18). This outbreak persisted into the 1980s and was subsequently found to be due to a unique plasmid or one of its closely related descendents that persisted in the hospital for at least 10 years (10, 11). Initially, up to 1980, the usage of amikacin was restricted and applied only in cases of gentamicin and tobramycin resistance. However, beginning in 1980, a new policy was instituted in which aminoglycosides were rotated, depending upon resistance in the hospital, using amikacin extensively if gentamicin resistance increased. We have previously reported our experience through 1984 with this practice (5), and the purpose of this report is to update our experience since 1984 with the rotational use of aminoglycoside antibiotics.

MATERIALS AND METHODS

From April 1980 through March 1990, aminoglycoside usage was continuously monitored from pharmacy records, and antibiotic resistance among gram-negative bacilli was monitored from microbiology laboratory records. The observation period was divided into distinct segments (Table 1) on the basis of the predominant aminoglycoside usage during that time, beginning with the period from April to July 1980 (period one), which is the baseline period during which gentamicin and tobramycin usage predominated. This was followed by a period from July 1980 until the end of August 1982 (period two), during which amikacin became the predominant aminoglycoside in use in the hospital. The third distinct period of observation was from September 1982 through the end of August 1983 (period three), a 1-year period during which gentamicin was reintroduced into the institution and was used at a reasonably high level. Beginning in September 1983 amikacin was once again reintroduced as the predominant aminoglycoside and was used through December 1985 (period four). The fifth and final alteration in usage began in January 1986, at which time gentamicin was once again reintroduced to the hospital, but was used at a modest level and gradually increased in percentage of usage over the period from 1986 through 1990 (period five). In late June 1988, patients and personnel moved to a new hospital building, and so resistance data for the new building were tabulated separately. Data were recorded for hospital location and infection site of each resistant organism.

Susceptibilities of gram-negative aerobic bacilli to the aminoglycosides were determined by the broth microdilution method (5). All aerobic and facultative gram-negative bacilli isolated from specimens submitted to the microbiology laboratory were screened for their susceptibilities to the study drugs. Only the first isolate of a species obtained during each

* Corresponding author.
month from each patient was used for analysis, unless the susceptibility of the organism changed by more than one dilution. Organisms were classified as resistant to gentamicin or tobramycin if the MIC was greater than 6 μg/ml, resistant to amikacin if the MIC was greater than 16 μg/ml, and resistant to netilmicin if the MIC was greater than 8 μg/ml. All susceptibility testing was performed in Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) ions. Over the course of the 10-year study, two different commercial microdilution systems, Microscan Systems Inc., Potomac, Md., and Microscan microdilution plates (American Microscan, Campbell, Calif.) and custom-manufactured plates made within the microbiology laboratory at the hospital were used. Introduction of the Microscan system late in 1982 was shown to significantly lower the geometric mean MICs for control strains of *Pseudomonas aeruginosa* and *Escherichia coli* when compared with those obtained with the previously used Microscan system (9). No effect on the susceptibilities of clinical isolates was noted, however, and the introduction of custom-manufactured plates in 1985 was not associated with a detectable change in the MICs for control isolates or the susceptibilities of clinical isolates. Statistical analysis of differences in resistance during the various usage periods was performed by a modified chi-square method (16), and rates of change and usage of aminoglycosides were analyzed by determining the standard error of the differences in slopes by using a T statistic (19).

### RESULTS

Summary data for each of the five study periods are given in Table 1. Although usage of aminoglycosides in this Medical Center (patient days per month) actually increased from 1980 to 1990, usage declined significantly over the past 5 years compared with usage in the first 5 years of the study (Fig. 1; \( P < 10^{-8} \)). Usage of tobramycin has been minimal in the hospital since the original baseline observation period in 1980 and constituted less than 2.5% of all usage after that period. Usage of netilmicin during the entire study was negligible. Hence, the primary aminoglycoside usage was divided between amikacin and gentamicin. The major change to amikacin usage was accompanied by a pharmacy policy requiring approval for use of gentamicin by the infectious disease service throughout periods two and four. The shift back to gentamicin was accomplished by placing both gentamicin and amikacin on open formulary without the need for approval for either one. The institution of a control policy for gentamicin resulted in a marked decrease in amikacin use during periods two and four (Table 1). During period three, gentamicin usage increased rapidly from 2.4 to 72% in 4 months, whereas in period five, the reintroduction of gentamicin proceeded at a more modest rate (from 1.6 to 79% over 15 months) and did not exceed the usage of amikacin until almost 1 year had passed (\( P = 0.048 \), rate of gentamicin usage change in period three versus that in period five). Since 1987, 75 to 90% of the quarterly aminoglycoside usage was gentamicin, with the balance primarily being amikacin.

Statistically significant changes in resistance to gentamicin and tobramycin occurred with each change in aminoglycoside policy with the exception of period five (Table 1). In period three, the reintroduction of gentamicin was rapid and emergence of resistance was also rapid; in period five, with a more gradual introduction of gentamicin and gradual reduction of amikacin usage, no significant rise in gentamicin resistance occurred. Overall, resistance to gentamicin during period five was 5.7%, which was essentially unchanged from that during period four. In contrast to the markedly changing resistance to gentamicin and tobramycin over time, there was no significant change in resistance to amikacin during any of the periods of monitoring, and resistance remained stable at 2.9 to 3.9% throughout the entire study. Resistance to netilmicin was measured only during period five and was 9% for all gram-negative bacilli, largely because of the 35% resistance of *P. aeruginosa*.

Aminoglycoside usage and percent resistance are shown

### TABLE 1. Aminoglycoside resistances of aerobic gram-negative bacilli and aminoglycoside usage during five periods of study from 1980 through 1990 at the Minneapolis Veterans Affairs Medical Center

<table>
<thead>
<tr>
<th>Study period</th>
<th>No. of months</th>
<th>No. of isolates</th>
<th>% Resistant</th>
<th>Aminoglycoside usage</th>
<th>% Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amikacin</td>
<td>Gentamicin</td>
<td>Tobramycin</td>
</tr>
<tr>
<td>One (baseline)</td>
<td>3</td>
<td>950</td>
<td>3.8</td>
<td>12.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Two (first amikacin)</td>
<td>26</td>
<td>6,235</td>
<td>3.2</td>
<td>6.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Three (first gentamicin)</td>
<td>12</td>
<td>2,849</td>
<td>3.9</td>
<td>9.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Four (second amikacin)</td>
<td>27</td>
<td>6,115</td>
<td>3.1</td>
<td>5.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Five (second gentamicin)</td>
<td>51</td>
<td>12,333</td>
<td>2.9</td>
<td>5.7</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*p < 0.001 compared with previous study period.

*p < 0.05 compared with previous study period.

FIG. 1. Aminoglycoside usage measured as patient days per quarter at the Minneapolis Veterans Affairs Medical Center from 1980 to 1990.
in Fig. 2 for period five to illustrate the possible effect of the patient move to a new hospital on resistance rates. A high rate of gentamicin usage was established by the first quarter of 1987, 18 months before the move took place, and did not change significantly after the move occurred. However, resistance to all three aminoglycosides was significantly lower in period five after the move than before the move ($P < 0.05$; Table 2). This trend toward lower resistance was evident for the year preceding the hospital move (Fig. 2) and continued during the 21 months after the move. The difference between resistance rates in the year prior to the move and the 21 months after moving was also statistically significant ($P < 0.05$).

Also given in Table 2 are the resistance rates by period of study for each of the major gram-negative bacillary species. The most significant decreases in resistance to gentamicin and tobramycin occurred among $P$. aeruginosa, Serratia spp. and Enterobacter spp. Currently, only resistance of $P$. aeruginosa to gentamicin exceeds $5\%$ among all of the bacterial species, and no species is more than $5\%$ resistant to amikacin or tobramycin. Resistance among all nonpseudomonal gram-negative bacilli is $1.5\%$ for amikacin, $2.7\%$ for gentamicin, and $2.8\%$ for tobramycin.

To analyze for aminoglycoside resistance by hospital location or service (data available through mid-1989), we divided the hospital locations into four major categories; intensive care units, urology service, surgical service, and medical service. The total number of intensive care unit beds was 42 in the old hospital and 43 in the new hospital; urology beds, 35 in the old hospital and 40 in the new hospital; surgical beds, 208 in the old hospital and 194 in the new hospital; and medical beds, 189 in the old hospital and 234 in the new hospital. No data on the total number of culture specimens submitted from each location were available, and the number of aminoglycoside-susceptible organisms isolated from each location was also unknown. The number of both gentamicin- and amikacin-resistant organisms found in the four hospital locations is shown in Fig. 3 by quarter of study. It is probable that a disproportionately high number of resistant isolates originated from the urology service, given their low number of inpatient beds compared with those in the medical and surgical services; but without data on the numbers of cultures submitted, the numbers of aminoglyco-

FIG. 2. Aminoglycoside resistance and usage during study period five in relation to the move to a new hospital building at the end of the second quarter of 1988. A, amikacin; G, gentamicin; T, tobramycin.

side-susceptible isolates cultured, and the numbers of specimens submitted from outpatients versus inpatients on the service, it is difficult to put these observations into perspective. Low numbers of resistant members of the family Enterobacteriaceae were evident for all locations in the latter half of the study. Higher numbers of resistant isolates originated from the surgical and urologic services in the first half of the study than from the medical service and intensive care units.

The sites from which patient specimens were obtained were also available through mid-1989. Aminoglycoside-resistant organisms were obtained from 891 urine specimens, 432 sputum specimens, 405 wounds (including decubitus ulcers), 85 body fluids (bile, ascites, pleural fluid, pericardial fluid, joint fluid, spinal fluid), 45 blood cultures, 13 stool specimens, 6 intravenous sites, and 17 miscellaneous or unknown sites. Since isolates were recorded only on the basis of first isolation of the same resistant species from a given patient in order to avoid duplication, it was not possible to determine all the sites from which aminoglycoside-resistant isolates were obtained, and the site list may be skewed by the relative rate of culturing of specimens from various sites and the rapidity with which organisms could be isolated and identified from the various sites by the microbiology laboratory.

**DISCUSSION**

Our experience with monitoring aminoglycoside usage and aminoglycoside resistance among gram-negative bacilli now encompasses 10 years and over 25,000 isolates. During that time, we noted an associated change in resistance with change in aminoglycoside usage policy. On two occasions, introduction of high-level amikacin use was associated with a significant decline in resistance to gentamicin and tobramycin, while reintroduction of gentamicin, on one occasion, resulted in a significant rise in resistance to both gentamicin and tobramycin. In contrast, during the fifth and last period of observation in which gentamicin was reintroduced at a modest level and gradually increased in percentage of usage, there has not been a significant increase in gentamicin or tobramycin resistance. During this final observation period, however, a move to a new hospital building occurred, and resistance rates following the move were significantly lower than they were before the move. We have no explanation for this observation, but a trend toward lower resistance was evident for a full year preceding the move (Fig. 2).

Throughout these changing periods of aminoglycoside usage, no significant increase in resistance to amikacin was observed. This is somewhat remarkable because we have documented within our organism population those bacteria, particularly Serratia spp., which contain the AAC-6' enzyme which is known to inactivate amikacin. Despite the presence of this enzyme in Serratia organisms, no increase and, in fact, a marked decline in the presence of Serratia organisms containing this enzyme was noted during periods of high amikacin use (8). Some institutions have noted increased resistance to amikacin with increased use of amikacin (2, 12, 17), while others have noted a decrease or no change in resistance (1, 14). Pooled data from 14 hospitals that instituted high-level (88%) amikacin usage indicated a small, but statistically significant ($P < 0.05$), increase in amikacin resistance from 1.4 to 1.7% of 95,000 isolates (5).

None of these hospitals individually detected a significant increase in amikacin resistance, however, an observation similar to our own long experience. Only one organism, $P$.
S. aureus, had significantly increased resistance (from 3.0 to 3.9%; \( P < 0.05 \)) in the 14-hospital study (2). These variable developments of resistance in response to amikacin use may depend upon the resistance mechanisms present in the gram-negative bacteria present in the individual hospitals.

At this time, we continue to use both gentamicin and amikacin in our institution, and currently, gentamicin usage predominates, with about 80 to 90% of aminoglycoside use being gentamicin and 10 to 20% being amikacin. This balance between the two aminoglycosides does not appear to have induced a return of the high-level gentamicin resistance that we experienced in the past. The primary enzyme mediating this resistance in our institution prior to 1983 was an ANT-2 enzyme encoded on an R plasmid which primarily was carried by Klebsiella, Enterobacter, and Serratia species (11). We were able to continue to document the presence of bacteria containing either the identical index plasmid or closely related plasmid species which were homologous to the original index plasmid through 1987. Since we moved to a new hospital building in 1988, we have not documented the presence of the index plasmid within environmental isolates of the new institution. At the time of the patient move, 14 of 590 patients from whom samples were obtained for culture were found to carry gentamicin-resistant members of the family Enterobacteriaceae in their stools, all of which were found to have plasmids which were homologous to our original index plasmid from 1975. However, these plasmids...
appeared to be different from our original index resistance plasmid, because they did not show the presence of the ANT-2 resistance gene when probed with a DNA sequence internal to this gene (3). Taken together, these data suggest that the original index plasmid is probably no longer present in large numbers of patients or the environment of our institution, but we have not systemically investigated large numbers of gentamicin-resistant organisms since 1988 to confirm this. Most gentamicin resistance at this time is found in *P. aeruginosa* and has not been associated with any enzymatic inactivation, but it is presumed to be due to a permeability barrier.

Aminoglycoside usage, as measured by patient days, rose from under 2,000 patient days per quarter in 1980 to well over 3,000 patients day per quarter in 1985 (Fig. 1). Since 1985, however, usage has declined significantly. These changes in aminoglycoside usage occurred as the daily hospital census declined linearly from 590 patients in 1980 to 465 in 1989, the last full year of the study. A declining census could explain the usage decline of the past 5 years, but it does not explain the rise in usage from 1980 to 1985. Substitution of other antimicrobial agents that are active against gram-negative bacilli and less toxic than aminoglycosides is another likely explanation for the recent decline in usage. Most of these alternative agents, such as ceftazidime, ceftriaxone, ceftriaxone, piperacillin, ticarcillin-clavulanate, imipenem-cilastatin, ciprofloxacin, and aztreonam, are on a restricted formulary and require telephone approval by the infectious disease service for use. We do not have accurate data on the usage of these agents in comparison with the usage of aminoglycosides, but we have noted increased requests for approvals for use in elderly patients and those with increased serum creatinine levels. Since the overall decline in aminoglycoside usage occurred almost entirely within period five of the surveillance study, this may have played an additional role in the lack of resistance development during this period of increased percentage of gentamicin usage.

Although the observed changes in aminoglycoside resistance following changes in aminoglycoside usage can only be termed associations and are not proven causes and effects, we remain impressed that there is a relationship between the availability of aminoglycosides within the hospital environment and the emergence of resistance. For these reasons, we have continued to follow our aminoglycoside usage patterns through 1990 and are monitoring resistance to the aminoglycosides by means of monthly susceptibility reports. At this time, it appears the balance we have achieved between the usage of gentamicin and amikacin is associated, in our institution, with an enviably low resistance to all aminoglycosides. Whether such a continued low resistance can be maintained by this usage policy will remain to be seen.

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