ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1992, p. 1596–1600
0066-4804/92/081596-05$02.00/0

Vol. 36, No. 8

Increased Resistance to Amikacin in a Neonatal Unit Following Intensive Amikacin Usage

IAN R. FRIEDLAND,[1]†* EVELYN FUNK,[1] MANIKANT KHOOSAL,[2] AND KEITH P. KLUGMAN[2]

Departments of Pediatrics[1] and Clinical Microbiology,[2] Baragwanath Hospital and the University of the Witwatersrand, Johannesburg, South Africa

Received 25 November 1991/Accepted 11 May 1992

Gram-negative isolates from blood and cerebrospinal fluid were monitored for 1 year before and for 1 year after the first-line aminoglycoside in a busy pediatric department was changed from gentamicin to amikacin. In the general pediatric wards, the switch to amikacin resulted in no change in resistance of nosocomial gram-negative infections to either amikacin (0% before and after) or gentamicin (23.9% [before] versus 26.5% [after]). In the neonatal unit, the switch to amikacin was followed by an outbreak of *Serratia* spp. that were commonly resistant to amikacin but susceptible to gentamicin. This outbreak abated spontaneously. In the year after the change in aminoglycoside usage, the resistance to amikacin of nosocomially acquired gram-negative infections increased from 7.6 to 27.7% ($P < 0.001$), and the resistance to gentamicin decreased from 71.2 to 60.2% ($P = 0.07$). The increase in amikacin resistance of gram-negative bacilli other than *Serratia* spp. has persisted for more than a year after the introduction of amikacin as the sole aminoglycoside. The different effects observed in the two sections of the pediatric department may be related to the more intensive usage of aminoglycosides in the neonatal unit.

The effect of predominant amikacin usage on susceptibility of gram-negative bacteria (GNB) to aminoglycosides is well documented (1, 5–7, 11, 13, 17, 19, 22–24, 28, 30). Those studies showed that frequent usage of amikacin usually results in no increase or only a slight increase in resistance to amikacin and a decrease in resistance to other aminoglycosides. However, in none of those studies were community- and hospital-acquired infections analyzed separately. Also, GNB isolated from all sites were included, and except in one study (6), invasive disease was not separated from colonization without invasion. Because all infections were combined, increases in amikacin resistance of nosocomial infections may have been "diluted" by the inclusion of large numbers of community-acquired isolates. Similarly, combining isolates from all sections of a hospital may mask significant changes in a specific area.

Further motivation for this study was the lack of information on antimicrobial resistance levels in relation to aminoglycoside usage in developing countries, where overcrowded hospitals with very high rates of patient turnover are common.

Because of increasing resistance of GNB to gentamicin, both the neonatal and general pediatric wards of our hospital changed to amikacin as the sole aminoglycoside for suspected GNB infections.

The purpose of this study was to record prospectively the effect of intensive amikacin usage on the aminoglycoside resistance patterns of invasive hospital-acquired GNB in a busy pediatric department.

MATERIALS AND METHODS

Patient population. Baragwanath is the major hospital serving Soweto, South Africa (population of 1 to 2 million). More than 20,000 babies are born at the hospital annually, and a further 12,000 are born in the surrounding clinics. The neonatal department has 47 intensive care beds (12 with ventilators) and 100 intermediate care beds and runs at nearly 100% capacity year-round. Annually, the neonatal intensive care unit admits 2,000 to 2,100 babies, and the intermediate care unit admits 2,900 to 3,000 patients. Approximately 5,000 more babies per year are seen in the neonatal unit in the maternity wards (low care ward).

The five general pediatric wards (surgery and oncology excluded) are housed in a different part of the hospital and annually admit almost 5,000 children <13 years old. There is only occasional transfer of patients and staff between the two sections.

Aminoglycoside usage. In the first year of the study period, gentamicin was the first-line aminoglycoside used in both the neonatal and general pediatric wards (gentamicin period). At that time, amikacin was reserved for nosocomial infections or for community-acquired (or congenitally acquired) infections resistant to or not responding to gentamicin. Other aminoglycosides have not been used in either the neonatal or the general wards. Because of increasing resistance of community-acquired and congenital GNB to gentamicin, the neonatal unit (in July 1989) and the general pediatric wards (in February 1990) introduced amikacin as the sole aminoglycoside (amikacin period). During this period, gentamicin was not used in either department. The percent aminoglycoside usage during the gentamicin period was calculated from pharmacy records.

Bacterial isolates and susceptibility. All blood and cerebrospinal fluid cultures taken in the pediatric department were reviewed daily for 12 months before and for 12 and 15 months in the general and neonatal wards, respectively, after the change in amikacin usage. Nosocomial infections were defined as those occurring >72 h after admission (or birth in the case of neonates) and not present on admission or at birth. Only aerobic GNB (excluding *Haemophilus* spp.) obtained from blood or cerebrospinal fluid are reported. Repeat isolates were not included in the analysis. Antimi-

* Corresponding author.
† Present address: Pediatric Infectious Disease, Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9063.

TABLE 1. Numbers of total isolates of community- and hospital-acquired GNB and of isolates resistant to aminoglycosides in general pediatric wards[a]

Period and organism	No. (%) of isolates							
	Community acquired				Nosocomially acquired			
	Total	Gentamicin resistant	Tobramycin resistant	Amikacin resistant	Total	Gentamicin resistant	Tobramycin resistant	Amikacin resistant
Gentamicin (Feb. 1989–Jan. 1990)								
Escherichia coli	43	3	3	0	14	1	1	0
Klebsiella spp.	9	4	4	1	14	9	7	0
Salmonella spp.	23	0	0	0	5	1	0	0
Others	38	6	4	1	13	0	0	0
All GNB	113	13 (11.5)	11 (9.7)	2 (1.8)	46	11 (23.9)	8 (17.4)	0 (0)
Amikacin (Feb. 1990–Jan. 1991)								
E. coli	41	0	0	0	8	1	1	0
Klebsiella spp.	25	9	9	1	13	6	5	0
Salmonella spp.	24	1	1	0	6	1	1	0
Others	34	1	1	1	7	1	1	0
All GNB	124	11 (8.9)	11 (8.9)	2 (1.6)	34	9 (26.5)	8 (23.5)	0 (0)

[a] There were no significant differences when total resistance values for each of the two periods were compared.

crobial susceptibilities were determined by Kirby-Bauer disk diffusion. Aminoglycoside resistance was confirmed periodically on randomly selected isolates by MIC determination according to National Committee for Clinical Laboratory Standards criteria (20). Gentamicin, tobramycin, and amikacin were the only aminoglycosides used in routine antimicrobial susceptibility testing.

Statistical analysis. Data obtained during the gentamicin period were compared with data from the amikacin period by the chi-square test with Yates' correction or Fisher's exact test, depending on sample size.

RESULTS

General pediatric wards. Amikacin and gentamicin were the only two aminoglycosides used, and 29% of children admitted during the study period received an aminoglycoside. Before the introduction of exclusive amikacin usage, gentamicin accounted for 78% of aminoglycoside usage. During the amikacin period, gentamicin was not used. The levels of resistance of GNB to amikacin, tobramycin, and gentamicin are shown in Table 1. No significant changes were observed following the change to exclusive amikacin usage.

Neonatal unit. Before the change to exclusive amikacin usage, gentamicin accounted for 87% of aminoglycoside usage. During the study period, 94% of babies admitted to the intensive care unit, 81% of babies admitted to the high care area, and 64% of babies admitted to the intermediate care units received an aminoglycoside. Babies in the low care wards only rarely received aminoglycosides. The percentages of neonates in each ward receiving aminoglycosides in the two periods of this study were similar.

The effect of exclusive amikacin usage on aminoglycoside resistance patterns is shown in Table 2. The overall amikacin resistance of nosocomial GNB increased from 7.6 to 27.7% ($P < 0.001$), and gentamicin resistance dropped from 71.2 to 60.2% ($P = 0.07$). *Klebsiella* spp. were the most frequently isolated nosocomial GNB (14 isolates from cerebrospinal fluid). The resistance of *Klebsiella* spp. to amikacin increased from 1.3 to 26.7% ($P < 0.001$), with no significant change in resistance to gentamicin. One amikacin-resistant *Klebsiella* isolate was found by DNA probe hybridization to possess the aminoglycoside 6′-*N*-acetyltransferase-I [AAC (6′)-I] gene.

In the year preceding the change to amikacin, a *Serratia* spp. was isolated only once. Within 3 months of the change, an outbreak of *Serratia* spp. occurred, with 25 episodes of nosocomial septicemia and four cases of meningitis in the space of 12 months. The evolution of this outbreak is shown in Fig. 1. Nine *Serratia* isolates (31%) were susceptible to gentamicin but resistant to amikacin. No other gentamicin-susceptible but amikacin-resistant organisms were isolated in either the neonatal or the general wards at any time during the study period. Despite an extensive search, no source for the *Serratia* outbreak was detected, and it abated with no specific intervention. The number of *Pseudomonas* isolates doubled during the amikacin period, but the resistance of *Pseudomonas* spp. to amikacin did not increase.

Amikacin-resistant isolates were also commonly resistant to broad-spectrum cephalosporins. Of 23 amikacin-resistant *Klebsiella* isolates, 20 (87%) were resistant to cefotaxime and 10 (43%) were resistant to ceftazidime. These multiply resistant infections were treated with an imipenem-cilastatin combination. In addition, imipenem was frequently used for suspected nosocomial infections not responding to amikacin. Convulsions were not noted with the usage of imipenem in 20 neonates (2 with meningitis).

Mortality rates. The fatality rate (within 1 week of a positive culture) among neonates infected with amikacin-resistant organisms (50%) was not significantly different from that of neonates infected with amikacin-susceptible organisms (43%, $P > 0.1$). Similarly, the fatality rate among bacteremic infants in the amikacin period (41%) was not statistically different from the rate in the gentamicin period (52%, $P > 0.1$).

DISCUSSION

This study differs from previous reports in four respects: (i) we considered only isolates known to be invasive, (ii) we separated hospital-acquired from community- or congeni-

TABLE 2. Numbers of total isolates of congenitally and nosocomially acquired GNB and of isolates resistant to aminoglycosides in neonatal wards

Period and organism	No. (%) of isolates							
	Congenitally acquired				Nosocomially acquired			
	Total	Gentamicin resistant	Tobramycin resistant	Amikacin resistant	Total	Gentamicin resistant	Tobramycin resistant	Amikacin resistant
Gentamicin (July 1988–June 1989)								
Klebsiella spp.	12	9	11	0	75	66	50	1
Serratia spp.	0				1	0	1	1
Pseudomonas spp.	0				11	8	10	6
Others	25	3	4	2	31	10	8	1
All GNB	37	12 (32.4)	15 (40.5)	2 (5.4)	118	84 (71.2)	69 (58.5)	9 (7.6)
Amikacin (July 1989–Sept. 1990)								
Klebsiella spp.	19	15	16	1	86	67[a]	56	23[b]
Serratia spp.	0				29	3	29	12
Pseudomonas spp.	0				26	24	24	11[a]
Others	29	9	9	1	51	21	20	7
All GNB	48	24[a] (50)	25[a] (52.1)	2[a] (4.2)	191	115[a] (60.2)	129[a] (67.5)	53[b] (27.7)

[a] Difference was not significant versus value for gentamicin period.
[b] $P < 0.001$ versus value for gentamicin period.

tally acquired infections, (iii) we compared the effects of similar changes in aminoglycoside usage in two sections of the same department in the same hospital, and (iv) during the amikacin period, no other aminoglycoside was used. Furthermore, we determined the effect on mortality of changes in antibiotic susceptibility observed in the neonatal unit.

This study illustrates the differing effects of increased amikacin usage that have previously been reported (1, 5–7, 9, 11, 13, 17–19, 22–24, 26, 28, 30). In the general pediatric wards, the exclusive use of amikacin was not associated with any appreciable change in resistance of nosocomial GNB to amikacin. Thirteen of 16 previous studies found little or no increase in amikacin resistance related to extended amikacin usage.

In contrast, the exclusive usage of amikacin in the neonatal unit was associated with a significant increase in resistance to this antibiotic, and there was a trend ($P = 0.07$) toward lower gentamicin resistance. It should be noted that amikacin resistance was 7.6% before the start of exclusive amikacin usage, indicating that resistant organisms were already present in the hospital environment. An outbreak of *Serratia* spp. occurred soon after the change in aminoglycoside usage. Many of the isolates of this organism were resistant to amikacin but susceptible to gentamicin. An increasing number of mechanisms by which GNB become resistant to amikacin are being described with the AAC(6′) mechanism being the most common (8, 13). *Serratia* strains are known to commonly produce AAC(6′) (12, 25). The gene coding for this enzyme has been shown to be carried on transposons which may conjugate with multiple plasmids and thereby be widely disseminated (15, 29). Nontransferable (i.e., chromosomally specified) AAC(6′) production by *Serratia* strains resulting in amikacin resistance without concomitant gentamicin resistance has also been reported (10). Recently, two other aminoglycoside-modifying enzymes, 4′-aminoglycoside nucleotidyltransferase and a 3′-phosphotransferase, have been reported to result in resistance to amikacin without concomitant resistance to gentamicin (4, 9).

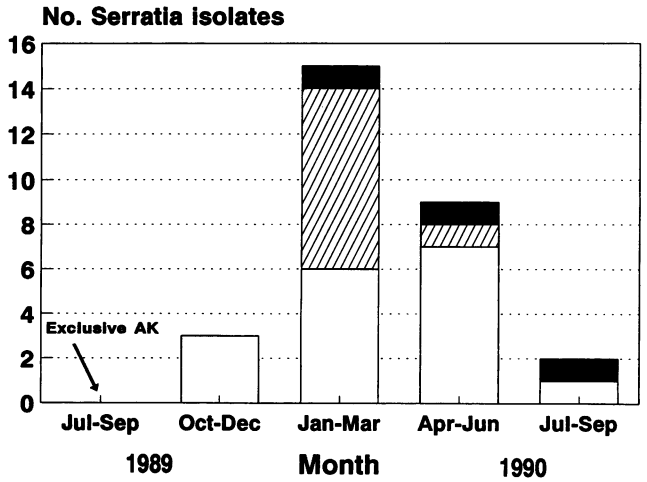

FIG. 1. Evolution of a *Serratia* outbreak in a neonatal unit. ▭, gentamicin and amikacin (AK) susceptible; ▨, gentamicin susceptible but AK resistant; ■, gentamicin and AK resistant.

Increased amikacin usage has been reported to result in increased bacterial production of AAC(6′) (13, 21), although Larson et al. (12) found decreased production of this enzyme with continued amikacin usage. The mechanism of amikacin resistance in one *Klebsiella* isolate from our neonatal unit was due to AAC(6′)-I. The mechanism of amikacin resistance among the *Serratia* isolates was not determined, but it is likely that AAC(6′) was responsible.

Outbreaks of *Serratia marcescens* are well documented, particularly outbreaks in neonatal units (14, 16, 27, 31). One reported outbreak was caused by netilmicin-resistant *S. marcescens* shortly after netilmicin had been introduced as the primary aminoglycoside (16). Outbreaks of amikacin-resistant but tobramycin-susceptible *Acinetobacter* spp. have also recently been reported (2). Since outbreaks due to various pathogens have previously occurred in our neonatal

unit (unpublished data), the *Serratia* outbreak described here may be unrelated to the change in aminoglycoside usage. However, it is likely that amikacin-resistant strains were selected through antibiotic pressure (15).

Increased resistance to amikacin in the neonatal unit was also noted in strains of *Klebsiella* spp., and the overall GNB resistance to amikacin increased to 27%. This is among the highest reported amikacin resistance levels for GNB other than *Pseudomonas* spp. or *Acinetobacter* spp. Although the increased amikacin resistance raised therapeutic dilemmas, there was no statistically significant increase in mortality from GNB infection during the amikacin period.

We have previously reported the high level of resistance to cephalosporins that developed in our neonatal unit (3). The high proportion of babies receiving antimicrobial agents (e.g., 94% receiving aminoglycosides in the neonatal intensive care unit) and the crowded conditions in this unit are likely to have contributed to the rapid development of high levels of resistance to various antimicrobial agents.

Resistance to both amikacin and the broad-spectrum cephalosporins necessitated the introduction of a new agent for the treatment of nosocomial infections resistant to or not responding to amikacin. Imipenem-cilastatin was the only agent available at our hospital to which GNB were uniformly susceptible and is currently the agent of choice for unresponsive nosocomially acquired infections in our neonatal unit. We will continue to monitor resistance patterns to assess the effect of this latest change in antimicrobial agent usage.

Data from the general pediatric wards and similar studies illustrate that increased resistance to amikacin usually does not develop following its use as a first-line aminoglycoside. However, an increase in amikacin usage (particularly in overcrowded conditions) may result in a significant increase in amikacin resistance of invasive hospital resident bacterial flora.

ACKNOWLEDGMENT

We thank George Miller of Schering Plough Research, Bloomfield, N.J., for performing the genetic analysis of the resistant *Klebsiella* strain.

REFERENCES

1. **Betts, R. F., W. M. Valenti, S. W. Chapman, T. Chonmaitree, G. Mowrer, P. Pincus, M. Messner, and R. Robertson.** 1984. Five-year surveillance of aminoglycoside usage in a university hospital. Ann. Intern. Med. **100**:219–222.
2. **Buisson, Y., G. Tran Van Nhieu, L. Ginot, P. Bouvet, H. Schill, L. Driot, and M. Meyran.** 1990. Nosocomial outbreaks due to amikacin-resistant tobramycin-sensitive *Acinetobacter* species: correlation with amikacin usage. J. Hosp. Infect. **15**:83–93.
3. **Funk, E., and S. Wainer.** 1990. Nosocomially acquired *Klebsiella* infection in an ICU—effect of short-term withdrawal of third-generation cephalosporins on cefotaxime resistance. Pediatr. Res. **27**:A271. (Abstract.)
4. **Gaynes, R., E. Groisman, E. Nelson, M. Casadaban, and S. A. Lerner.** 1988. Isolation, characterization, and cloning of a plasmid-borne gene encoding a phosphotransferase that confers high-level amikacin resistance in enteric bacilli. Antimicrob. Agents Chemother. **32**:1379–1384.
5. **Gerding, D. N., T. A. Larson, R. A. Hughes, M. Weiler, C. Shanholtzer, and L. R. Peterson.** 1991. Aminoglycoside resistance and aminoglycoside usage: ten years of experience in one hospital. Antimicrob. Agents Chemother. **35**:1284–1290.
6. **Hammond, J. M. J., P. D. Potgieter, A. A. Forder, and H. Plumb.** 1990. Influence of amikacin as the primary aminoglycoside on bacterial isolates in the intensive care unit. Crit. Care Med. **18**:607–610.
7. **Hesseling, P. B., P. L. van der Merwe, and R. Robson.** 1990. A prospective study of the long term use of amikacin in a paediatrics department: indications, administration, side effects, bacterial isolates, and resistance. S. Afr. Med. J. **78**:192–195.
8. **Jacoby, G. A., and G. L. Archer.** 1991. New mechanisms of bacterial resistance to antimicrobial agents. N. Engl. J. Med. **324**:601–612.
9. **Jacoby, G. A., M. J. Blaser, P. Santanam, H. Hächler, F. H. Kayser, R. S. Hare, and G. H. Miller.** 1990. Appearance of amikacin and tobramycin resistance due to 4′-aminoglycoside nucleotidyltransferase [ANT(4′)-II] in gram-negative pathogens. Antimicrob. Agents Chemother. **34**:2381–2386.
10. **John, J. F., Jr., W. F. McNeill, K. E. Price, and P. A. Kresel.** 1982. Evidence for a chromosomal site specifying amikacin resistance in multiresistant *Serratia marcescens*. Antimicrob. Agents Chemother. **21**:587–591.
11. **King, J. W., M. C. White, J. R. Todd, and S. A. Conrad.** 1992. Alterations in the microbial flora and in the incidence of bacteremia at a university hospital after adoption of amikacin as the sole formulary aminoglycoside. Clin. Infect. Dis. **14**:908–915.
12. **Larson, T. A., C. R. Garrett, and D. N. Gerding.** 1986. Frequency of aminoglycoside 6′-*N*-acetyltransferase among *Serratia* species during increased use of amikacin in the hospital. Antimicrob. Agents Chemother. **30**:176–178.
13. **Levine, J. F., M. J. Maslow, R. E. Leibowitz, A. A. Pollock, B. A. Hanna, S. Schaefler, M. S. Simberkoff, and J. J. Rahal.** 1985. Amikacin-resistant gram-negative bacilli: correlation of occurrence with amikacin use. J. Infect. Dis. **151**:295–300.
14. **Lewis, D. A., P. M. Hawkey, J. A. Watts, D. C. Speller, R. J. Primavesi, P. J. Fleming, and T. L. Pitt.** 1983. Infection with netilmicin-resistant *Serratia marcescens* in a special care baby unit. Br. Med. J. **287**:1701–1705.
15. **Mayer, K. H.** 1986. Review of epidemic aminoglycoside resistance worldwide. Am. J. Med. **80**(Suppl. 6B):56–64.
16. **Montanaro, D., G. M. Grasso, I. Annino, N. De Ruggiero, A. Scarcella, and F. Schioppa.** 1984. Epidemiological and bacteriological investigation of *Serratia marcescens* epidemic in a nursery and in a neonatal intensive care unit. J. Hyg. **93**:67–78.
17. **Moody, M. M., C. A. De Jongh, S. C. Schimpff, and G. L. Tillman.** 1982. Long-term amikacin use: effects on aminoglycoside susceptibility of gram-negative bacilli. JAMA **248**:1199–1202.
18. **Moody, M. M., V. M. Young, S. C. Schimpff, et al.** 1979. In vitro antibacterial activity of four aminoglycosides and two semisynthetic penicillins on fresh clinical isolates from cancer patients. Eur. J. Cancer **15**:23–33.
19. **Muscato, J. J., D. W. Wilbur, J. J. Stout, and R. A. Fahrlender.** 1991. An evaluation of the susceptibility patterns of gram-negative organisms isolated in cancer centres with aminoglycoside usage. J. Antimicrob. Chemother. **27**(Suppl. C):1–7.
20. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS publication M7-A2, vol. 10, no. 8. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **Price, K. E., P. A. Kresel, L. A. Farchione, S. B. Siskin, and S. A. Karpow.** 1981. Epidemiological studies of aminoglycoside resistance in the U.S.A. J. Antimicrob. Chemother. **8**(Suppl.): 89s–105s.
22. **Price, K. E., and S. B. Siskin.** 1984. Ecological consequences of the intensive use of amikacin. Chemioterapia **3**(Suppl.):12–19.
23. **Ruiz-Palacios, G. M., S. Ponce de Leon, J. Sifuentes, S. Ponce de Leon, J. J. Calva, F. Huazano, C. Ontiveros, F. Ojeda, and M. Bobadilla.** 1986. Control of emergence of multi-resistant gram-negative bacilli by exclusive use of amikacin. Am. J. Med. **80**(Suppl. 6B):71–75.
24. **Saavedra, S., D. Vera, and C. H. Ramirez-Ronda.** 1986. Susceptibility of aerobic gram-negative bacilli to aminoglycosides: effects of 45 months of amikacin as first-line aminoglycoside therapy. Am. J. Med. **80**(Suppl. 6B):65–70.
25. **Shimizu, K., T. Kumada, W.-C. Hsieh, H.-Y. Chung, Y. Chong, R. S. Hare, G. H. Miller, F. J. Sabatelli, and J. Howard.** 1985. Comparison of aminoglycoside resistance patterns in Japan,

Formosa, and Korea, Chile, and the United States. Antimicrob. Agents Chemother. **28**:282–288.
26. **Shulman, S. T., and R. Yogev.** 1985. Treatment of pediatric infections with amikacin as first-line aminoglycoside. Am. J. Med. **79**(Suppl. 1A):43–50.
27. **Smith, P. J., D. S. Brookfield, D. A. Shaw, and J. Gray.** 1984. An outbreak of *Serratia marcescens* infections in a neonatal unit. Lancet **i**:151–153.
28. **Vanhoof, R., J. M. Hubrechts, H. J. Nyssen, and E. Roebben.** 1988. Three-year survey of amikacin use and aminoglycoside resistance in a general hospital in Belgium. Eur. J. Clin. Microbiol. Infect. Dis. **7**:183–185.
29. **Voigt, W., H. Tschape, and H. Heier.** 1987. Amikacin-acetyltransferase (AAC-6′/I) encoded by an IncH2 plasmid (pIE866) in *Escherichia coli* strains. J. Basic Microbiol. **27**:461–464.
30. **Young, E. J., C. M. Sewell, M. A. Koza, and J. E. Clarridge.** 1985. Antibiotic resistance patterns during aminoglycoside restriction. Am. J. Med. Sci. **290**:223–227.
31. **Zaidi, M., J. Sifuentes, M. Bobadilla, D. Moncada, and S. Ponce de Leon.** 1989. Epidemic of *Serratia marcescens* bacteremia and meningitis in a neonatal unit in Mexico City. Infect. Control Hosp. Epidemiol. **10**:14–20.