Clinical and laboratory study of tobramycin in the treatment of infections due to gram-negative organisms

T.J. MARRIE, MD; M.J. GURWITH, MD, FRCP[C]; A.R. RONALD, MD, FRCP[C]; H.G. STIVER, MD, FRCP[C]; B. LANK, RN; L. FOX, RT

Tobramycin, an aminoglycoside antibiotic, was used to treat 52 infections due to gram-negative organisms in 51 patients. Complicated urinary tract infections, bacteremia and pyelonephritis accounted for 80% of the infections.

The rate of immediate satisfactory response was 79%. During therapy with tobramycin, resistant organisms emerged in four patients — two *Pseudomonas aeruginosa* and two *Escherichia coli* strains. There were four superinfections with tobramycin-resistant *Providencia* sp. In four seriously ill patients the serum creatinine concentration increased 1 mg/dL or more; in three the increase was transient. No auditory toxicity was noted in the 19 patients in whom serial audiograms were made.

In vitro testing of isolates from these patients showed that tobramycin and gentamicin had equal activity against Enterobacteriaceae. Tobramycin was two to four times more active against susceptible *P. aeruginosa*.

La tobramycine, un antibiotique aminoglycosidique, a été utilisée pour traiter 52 infections à bactéries gram-négatif survenant chez 51 patients. Des infections des voies urinaires avec complications, des bactérémies et des pyélonéphrites ont constitué 80% de ces infections.

Le taux des résultats satisfaisants immédiats a été de 79%. Pendant le traitement à la tobramycine, des micro-organismes résistants sont apparus chez quatre patients — deux souches de *Pseudomonas aeruginosa* et deux d'*Escherichia coli*. Quatre cas de surinfections à *Providencia* sp. résistantes à la tobramycine ont été rencontrés. Chez quatre patients gravement malades le taux de créatinine sérique a augmenté 1 mg/dL ou plus; chez trois d'entre eux cette augmentation a été passagère. Aucun signe d'ototoxicité n'a été décelé chez les 19 patients chez qui des audiogrammes ont été pratiqués.

L'antibiogramme des prélèvements faits chez ces patients a révélé que la tobramycine et la gentamicine étaient d'égale activité contre les Enterobacteriaceae. La tobramycine était deux à quatre fois plus active contre les souches sensibles de *P. aeruginosa*.

Tobramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius*.[1] It is slightly more active than gentamicin against Enterobacteriaceae and substantially more active in vitro against isolates of *Pseudomonas aeruginosa*.[2-5] The pharmacodynamics of tobramycin are almost identical to those of gentamicin with the same dose and route of administration.[6,7]

This paper reports the clinical efficacy and toxicity of tobramycin in 52 infections due to gram-negative organisms in 51 patients. Tobramycin activity against isolates from these patients is compared in vitro with that of gentamicin, amikacin and kanamycin.

Patients and methods

Patients at either the Health Sciences Centre or St. Boniface General Hospital, Winnipeg who were suspected or proven to have serious infections due to gram-negative organisms were entered into the study. Verbal informed consent was obtained before institution of therapy with tobramycin. Patients were considered cured if they became afebrile, all signs of infection cleared and follow-up cultures showed eradication of the original pathogen.

Antimicrobial susceptibility was assessed by the standardized disc-testing method with a disc containing 10 μg of tobramycin. The minimal inhibitory concentration (MIC) was determined by an agar-plate dilution method. The organisms to be tested were grown for 3 hours in Mueller–Hinton broth, then diluted to a concentration of 10^6/mL. With a Steers replicator approximately 5×10^3 organisms were inoculated onto Mueller–Hinton agar medium containing serial twofold dilutions of each of the antimicrobial agents, the concentrations ranging from 128 to 0.25 μg/mL.[8] The MIC was recorded as the lowest concentration of antibiotic with which three or fewer colonies grew after overnight incubation. An MIC of 8 μg/mL or more was considered to indicate tobramycin resistance, and an MIC of 4 μg/mL or less or a zone of inhibition of 20 mm in diameter on disc sensitivity testing was considered to indicate tobramycin sensitivity. Pyocine typing was carried out on most *P. aeruginosa* isolates.[9]

Tobramycin serum concentrations were measured by the agar-well diffusion method with *Bacillus subtilis* as the assay organism. Serum was usually obtained on days 2 and 5 of therapy. After intravenous administration of tobramycin the first serum sample was obtained 1 hour following cessation of infusion. After intramuscular administration serum was obtained at 1, 2, 3, 4 and 6 hours, and just prior to the next dose. The drug was given by intravenous infusion to 28 patients and by intramuscular injection to 23; 19 were treated with 1.5 mg/kg *q8h*, 17 with 1.0 mg/kg *q8h* and the remainder with larger doses because of inadequate serum concentrations or lower doses because of renal insufficiency.

Complete blood counts and measurement of concentrations of blood urea nitrogen, serum creatinine, glutamic oxaloacetic transaminase, lactic dehydrogenase and alkaline phosphatase were done at the beginning, every 3 or 4 days during and at the end of treatment. Whenever possible creatinine clearance was estimated at the beginning and end of therapy.

Serial audiograms were made before and during therapy in 19 patients with a portable model 9D Beltone audiometer.

Results and case reports

The 51 patients, 26 females and 25 males, received 52 courses of treatment with tobramycin. Therapy was given for a median of 9.0 days (range, 4 to 37 days; 20 to 37 days in six patients). The median age was 56 years (range, 8 to 91 years). The overall rate of immediate satisfactory response was 79%; superinfections developed in seven of the patients with an initial favourable response (Table I). In 10 patients the infection failed to respond to therapy.

Complicated urinary tract infection was diagnosed in 17 patients. Six of these had a neurogenic bladder and three others had chronic renal disease. The nosocomial nature of this infection was reflected in the type of organisms isolated (Table II). In four patients the infecting organism was not eradicated. In one of these, a patient with chronic

From the departments of medical microbiology and medicine, University of Manitoba, and the infectious disease services, Health Sciences Centre and St. Boniface General Hospital, Winnipeg

Reprint requests to: Dr. T.J. Marrie, Department of medicine, Victoria General Hospital, Halifax, NS B3H 2Y9

Table I—Summary of results of tobramycin therapy

Infection	No. (and %) of infections			
	Total no.	Immediate satisfactory response	Superinfection	Failure
Complicated urinary tract infection	17	13	4	4
Bacteremia	13	10	2	3
Acute pyelonephritis	13	11	—	2
Pneumonia	3	2	—	1
Bronchitis	1	1	1	—
Lung abscess	1	—	—	1
Septic arthritis	1	1	—	—
Perichondritis	1	1	—	—
Ascending cholangitis	1	1	—	—
Meningitis	1	1	—	—
Total	52	41 (79)	7 (13)	11 (21)

Table II—Organisms isolated from 17 patients with complicated urinary tract infection

Organism	No. of isolates
Pseudomonas aeruginosa	13
Escherichia coli	3
Klebsiella sp.	2
Proteus morganii	1
Enterobacter sp.	1
Providencia sp.	1

glomerulonephritis, *P. aeruginosa* resistant to tobramycin was isolated from prostatic secretions following removal of the patient's kidneys in preparation for renal transplantation. In the remaining 13 patients the urine was temporarily sterilized but became reinfected in 4 with a tobramycin-resistant *Providencia* sp.

Of the 13 patients with bacteremia 9 (70%) were cured. Ten were treated with tobramycin only. The source of the bacteremia was the urinary tract in eight, the biliary tree in two and unknown in three who had granulocytopenia as the result of treatment of acute leukemia. In one patient two organisms, *P. aeruginosa* and *Escherichia coli,* were isolated from the blood stream. *E. coli* was isolated from six patients, *P. aeruginosa* from four, *Klebsiella* sp. from three and *Proteus mirabilis* from one. Three of the four patients whose condition did not improve with antimicrobial therapy had acute leukemia. The fourth had pyelonephritis resulting in renal failure; during treatment with tobramycin the *E. coli* in the urine became resistant to tobramycin, the MIC increasing from 2 to 16 μg/mL, while it remained sensitive to 2 μg/mL of gentamicin.

Twelve patients had 13 episodes of acute pyelonephritis. Intravenous pyelograms were abnormal in nine. *P. aeruginosa* was isolated from the urine on five occasions, *E. coli* on six and *Klebsiella* sp. and *Citrobacter* sp. once each. Therapy eradicated the infecting organism in 11 instances (85%). Follow-up cultures in 10 patients 4 to 6 weeks later were negative. One patient relapsed within 4 weeks, and the other patient, who had chronic renal failure, had persisting infection with tobramycin-sensitive *E. coli.*

Pneumonia was treated with tobramycin in two patients and with tobramycin and carbenicillin in one patient. The latter, an 85-year-old man, died, and at autopsy *P. aeruginosa* pneumonia was discovered. The other two patients were cured: in one both *Klebsiella* sp. and *Serratia* sp. were grown from a lung biopsy; in the other *P. aeruginosa* and *Enterobacter* sp. were isolated from the sputum.

One patient with bronchitis due to *Enterobacter* sp. was successfully treated with tobramycin in an effort to wean her from assisted ventilation. Ten days after completion of therapy tobramycin-resistant *Providencia* sp. was cultured from the urine.

Lung abscess, septic arthritis, perichondritis, ascending cholangitis and meningitis occurred in one patient each and were treated with tobramycin and carbenicillin. The case histories of these patients are summarized below.

Case 1

A lung abscess developed in an 8-year-old girl with Fanconi's anemia and primordial dwarfism. Culture of abscess material obtained by percutaneous aspiration grew *P. aeruginosa* resistant to tobramycin, gentamicin, amikacin, kanamycin and carbenicillin. In vitro synergy studies suggested that tobramycin and carbenicillin were the most active combination. A very large dose of tobramycin (4 mg/kg) was required for an adequate blood concentration (6 to 8 μg/mL). The lung abscess did not improve and the patient died as a result of recurrent pneumothorax.

Case 2

P. aeruginosa peritonitis and bacteremia occurred during peritoneal dialysis in a 68-year-old man with Guillain-Barré syndrome and acute renal failure. Three weeks following treatment with carbenicillin and gentamicin pain, erythema and fluctuance of the left wrist developed; culture of aspirated fluid grew *P. aeruginosa.* Combined therapy with tobramycin and carbenicillin for 22 days resulted in cure.

Table III—Mean serum concentrations after intravenous infusion of tobramycin in 22 patients

Time after infusion (h)	Dose and serum concentration (μg/mL) of tobramycin			
	1.5 mg/kg		1.0 mg/kg	
	No.*	Mean ± SD	No.*	Mean ± SD
1	3	7.1 ± 0.5	—	—
2	6	4.7 ± 1.3	4	4.1 ± 3.0
3	9	3.5 ± 1.3	3	3.2 ± 1.1
4	8	2.9 ± 1.4	4	1.7 ± 0.8
6	6	2.5 ± 1.2	4	1.2 ± 0.8
7½	7	1.8 ± 0.9	3	0.7 ± 0.5

*No. of determinations.

Table IV—Mean serum concentrations after intramuscular injection of tobramycin in 22 patients

Time after infusion (h)	Dose and serum concentration (μg/mL) of tobramycin			
	1.5 mg/kg		1.0 mg/kg	
	No. *	Mean ± SD	No.*	Mean ± SD
1	4	5.7 ± 1.3	8	3.0 ± 0.8
2	4	3.8 ± 0.8	8	2.1 ± 0.7
3	—	—	6	1.6 ± 0.8
4	3	2.6 ± 0.8	8	1.4 ± 0.7
6	2	1.3 ± 0.2	8	0.9 ± 0.5
7½	5	1.0 ± 0.8	8	0.6 ± 0.4

*No. of determinations.

Case 3

Perichondritis developed in a 17-year-old girl secondary to otitis externa; *P. aeruginosa, P. mirabilis* and *Streptococcus faecalis* were isolated from the ear discharge. Treatment with tobramycin and carbenicillin for 17 days was successful.

Case 4

Ascending cholangitis occurred in a 20-year-old man with a choledochal cyst. Culture of tissue from a percutaneous liver biopsy grew *P. aeruginosa, Klebsiella* sp. and *Staphylococcus* epidermidis. Cure was achieved with 20 days of therapy with tobramycin, carbenicillin and cefazolin.

Case 5

Meningitis due to *Serratia* sp. followed repair of a cerebral arteriovenous fistula in a 27-year-old man. Treatment with tobramycin and carbenicillin for 21 days resulted in cure in spite of a cerebrospinal fluid concentration of tobramycin of only 0.5 μg/mL 4 hours after intravenous infusion of the antibiotic.

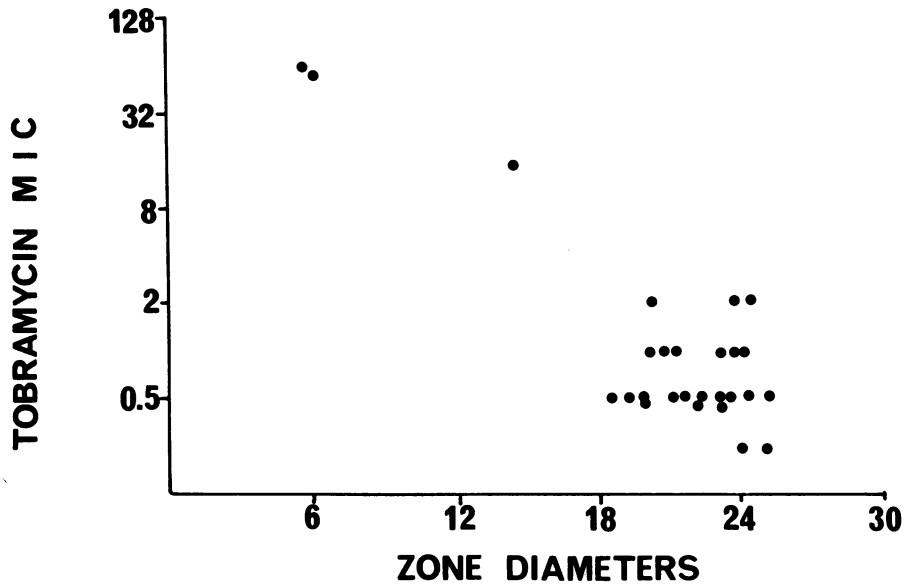

FIG. 1—Minimal inhibitory concentration (MIC; μg/mL) v. zone diameter (mm) with sensitivity-testing discs containing 10 μg of tobramycin for 27 *Pseudomonas aeruginosa* strains.

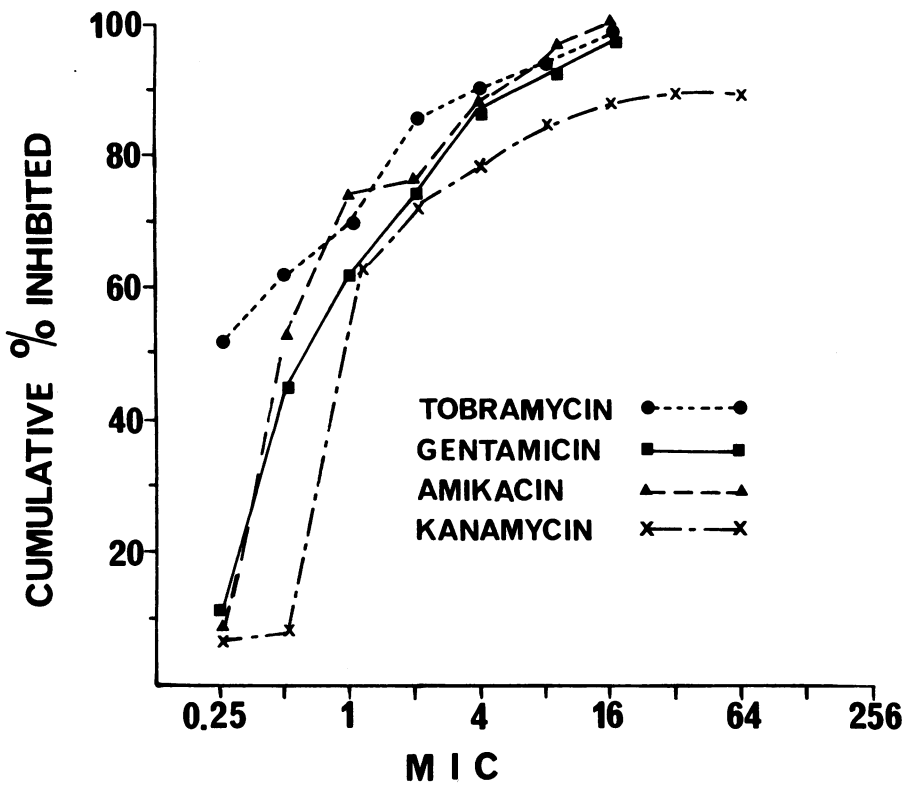

FIG. 2—Cumulative percent inhibition by tobramycin, gentamicin, amikacin and kanamycin of 49 Enterobacteriaceae isolates.

Tobramycin serum concentrations

Tobramycin concentrations in the serum were determined several times during therapy in 30 patients; for the 22 with normal renal function the mean values obtained by the intravenous and intramuscular routes with doses of 1.5 and 1.0 mg/kg are given in Tables III and IV.

Toxicity

No vestibular disturbances were noted and in the 19 patients in whom serial audiograms were made the readings were unchanged during therapy. In four seriously ill patients the serum creatinine concentration increased by 1 mg/dL or more. In three the increase was transient. The fourth was treated with tobramycin for *P. aeruginosa* urinary tract infection. Her course was complicated by septic thrombophlebitis of an antecubital vein due to *S. aureus*; this infection was treated with methicillin, which was discontinued when the serum creatinine value increased from 1.4 to 4.0 mg/dL. The highest serum concentration of tobramycin was 9 μg/mL. At autopsy the renal tubules showed marked autolysis and there was hypertrophy of the medium-sized renal arterioles. There was no interstitial nephritis.

Serial hematologic and hepatic studies showed no evidence of toxicity attributable to tobramycin.

In vitro studies of isolates from these patients

The correlation for 27 *P. aeruginosa* strains between the zone of inhibition surrounding the 10-μg tobramycin disc and the MIC is shown in Fig. 1.

The MICs of tobramycin, gentamicin, amikacin and kanamycin for 49 isolates of Enterobacteriaceae and 30 strains of *P. aeruginosa* are compared in Figs. 2 and 3. Tobramycin, gentamicin and amikacin had similar in vitro activity against the Enterobacteriaceae. At a concentration of 2 μg/mL tobramycin inhibited 83% of the *P. aeruginosa* strains, whereas gentamicin inhibited only 43%. At a concentration of 8 μg/mL the proportion of strains inhibited by gentamicin increased to 87%, while the proportion inhibited by tobramycin did not change.

Discussion

We found tobramycin to be effective against 79% of a variety of infections due to gram-negative organisms. Blair and colleagues[10] gave 29 courses of tobramycin to 25 patients with serious *P. aeruginosa* infections and obtained a satisfactory response in 52% of the patients. Jaffe and associates[2] treated

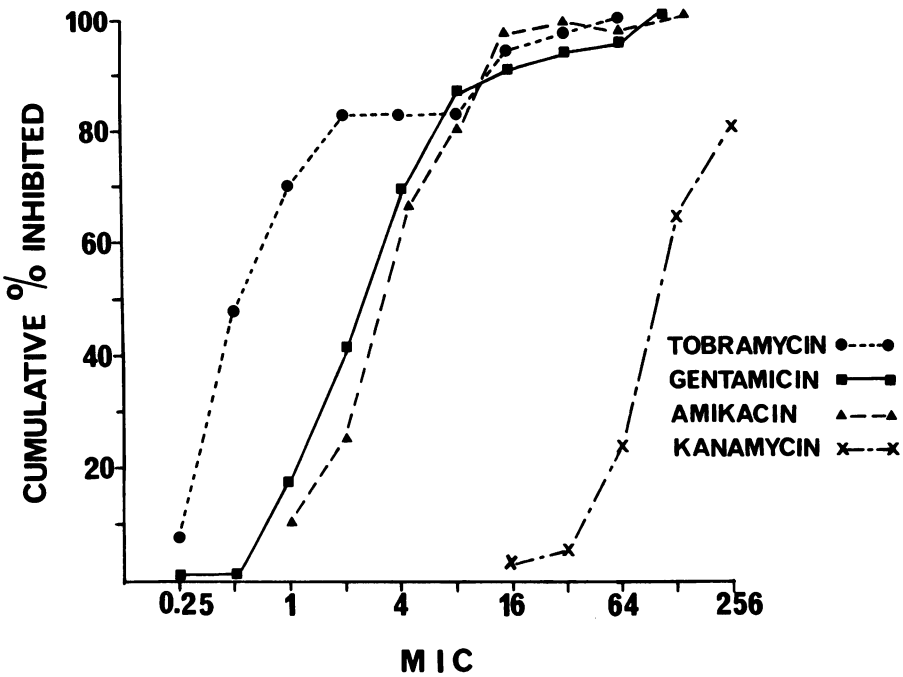

FIG. 3—Cumulative percent inhibition by tobramycin, gentamicin, amikacin and kanamycin of 30 strains of *P. aeruginosa*.

15 patients, 10 of whom had gram-negative bacteremia, with tobramycin; 12 of the 15 (80%) were cured of their infection. Valdivieso and colleagues[11] used tobramycin to treat 82 febrile episodes in 73 cancer patients; 44 episodes (54%) responded to this therapy. Our response rate of 79% in patients with gram-negative bacteremia compares favourably with the results obtained in the treatment of such infections with gentamicin or with kanamycin plus polymyxin.[12-15]

The peak tobramycin concentrations in the serum following doses of 1.0 and 1.5 mg/kg were generally lower than those reported by Jaffe and associates[2] and Blair and colleagues.[10] There was substantial interpatient variation in the peak value in patients with normal renal function. Similar variation has been found with gentamicin.[16] Most gentamicin toxicity has been associated with serum concentrations of 8 to 12 μg/mL or more;[17,18] since the pharmacokinetics and toxicity of gentamicin and tobramycin are similar, comparable concentrations of tobramycin might be expected to be toxic. Only 8% of our patients had an increase in their serum creatinine concentration of 1 mg/dL or more during tobramycin therapy. In three the increase was transient and the fourth received another nephrotoxic drug and was seriously ill. No ototoxicity was demonstrable by means of serial audiograms. Experiments with guinea pigs have shown that the cochlear toxicity of tobramycin is less than that of gentamicin.[19]

In vitro studies showed an excellent correlation between the diameter of the zone of inhibition obtained with the 10-μg tobramycin disc and the MIC for 27 *P. aeruginosa* isolates. A zone diameter of 18 mm or more correlated with an MIC of 2 μg/mL or less. Laxer, Mackay and Marks[20] found that a zone diameter of 14 mm or more predicted susceptibility to 5 μg/mL of tobramycin. Susceptible strains of *P. aeruginosa* were inhibited by concentrations of tobramycin that were two to four times less than those of gentamicin or amikacin.

In one study[21] 99% of 500 isolates of *P. aeruginosa* were susceptible to 4 μg/mL of tobramycin; in another study[3] 98% of 150 such isolates were susceptible to that concentration of tobramycin. Before tobramycin was used at our hospital 98% of 327 isolates of *P. aeruginosa* and essentially all Enterobacteriaceae had been found to be susceptible to 3.1 μg/mL of the drug. However, only 27 of 30 strains of *P. aeruginosa* isolated from tobramycin-treated patients in this study were susceptible to 4 μg/mL of tobramycin. The three patients with resistant organisms had received gentamicin within 4 weeks of institution of tobramycin therapy. Four patients had tobramycin-resistant *Providencia* sp. isolated following treatment with tobramycin.

Conclusion

We found tobramycin to be effective in the treatment of infections due to gram-negative organisms. The two- to fourfold lower MICs of tobramycin for susceptible *P. aeruginosa* isolates may be clinically advantageous, but the early emergence of resistant strains portends concern for its eventual role.

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References

1. KOCH KF, RHOADES JA: Structure of nebramycin factor 6, a new aminoglycoside antibiotic, *Antimicrob Agents Chemother, 1970:* p 309, 1971
2. JAFFE G, RAVRELY W, MEYER BR, et al: Clinical study of the use of the new aminoglycoside tobramycin for therapy of infections due to gram-negative bacteria. *Antimicrob Agents Chemother* 5: 75, 1974
3. LOCKWOOD WR, LAWSON LA: Studies on the susceptibility of 150 consecutive clinical isolates of *Pseudomonas aeruginosa* to tobramycin, gentamicin, colistin, carbenicillin and five other antimicrobials. *Antimicrob Agents Chemother* 4: 281, 1973
4. DIENSTAG J, NEU HC: In vitro studies of tobramycin and aminoglycoside antibiotics. *Antimicrob Agents Chemother* 1: 41, 1972
5. BODEY GP, STEWARD D: In vitro studies of tobramycin. *Antimicrob Agents Chemother* 2: 109, 1972
6. HORIKOSHI W, VALDIVIESO M, BODEY GP: Clinical pharmacology of tobramycin. *Am J Med Sci* 266: 453, 1974
7. REGAMEY C, GORDON RC, KIRLEY WMM: Comparative pharmacokinetics of tobramycin and gentamicin. *Clin Pharmacol Ther* 14: 396, 1973
8. STEERS E, FOLTZ EL, GRAVES BS: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot Chemother* 9: 307, 1959
9. GILLIES RR, GOVAN JRW: Typing of *Pseudomonas pyocyanea* by pyocine production. *J Pathol Bacteriol* 91: 339, 1966
10. BLAIR DC, FEKETY FR JR, BRUCE B, et al: Therapy of *Pseudomonas aeruginosa* infections with tobramycin. *Antimicrob Agents Chemother* 8: 22, 1975
11. VALDIVIESO M, HORIKOSHI N, RODRIGUEZ V, et al: Therapeutic trials with tobramycin. *Am J Med Sci* 268: 149, 1974
12. COX CE, HARRISON LH: Comparison of gentamicin and polymyxin B–kanamycin in therapy of bacteremia due to gram-negative bacilli. *J Infect Dis* 124 (suppl): S156, 1971
13. MCHENRY MC, GAVAN TL, VANOMMEN RA, et al: Therapy with gentamicin for bacteremic infections: results with 53 patients. Ibid, p S164
14. BODEY GP, MIDDLEMAN E, UMSAWASDI T, et al: Intravenous gentamicin therapy for infections in patients with cancer. Ibid, p S174
15. HOLLOWAY WJ, TAYLOR WA: Gentamicin and kanamycin in the treatment of gram-negative sepsis: a comparative study. Ibid, p S180
16. SIBER GR, ECHEVERRIA P, SMITH AL, et al: Pharmacokinetics of gentamicin in children and adults. *J Infect Dis* 132: 637, 1975
17. FALCO FG, SMITH HM, ARCIERI GM: Nephrotoxicity of aminoglycosides and gentamicin. *J Infect Dis* 119: 406, 1969
18. SIMON VK, MOSINGER EU, MALERCZY V: Pharmacokinetic studies of tobramycin and gentamicin. *Antimicrob Agents Chemother* 3: 445, 1973
19. FEDERSPIL P: Histological investigations of the ototoxicity of gentamicin and tobramycin. *Drug Res* 23: 1739, 1973
20. LAXER RM, MACKAY E, MARKS MI: Antibacterial activity of tobramycin against gram-negative bacteria and the combination of ampicillin/tobramycin against *E. coli*. *Chemother* 21: 90, 1975
21. DUNCAN IBR, PENNER JL: Comparative activity of tobramycin and gentamicin against Pseudomonas, Proteus and Providencia species. *Can Med Assoc J* 113: 29, 1975