STUDY ON *IN VITRO* ACTIVITY OF THE ANTIBIOTICS TOBRAMYCIN AND GENTAMICIN AGAINST *PSEUDOMONAS AERUGINOSA* CLINICAL STRAINS*,**

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(Received for publication January 8, 1973)

The *in vitro* activity of the aminoglycosides tobramycin and gentamicin was determined through a study using 100 clinical *Pseudomonas aeruginosa* and 3 international standard strains. Tobramycin was clearly more active against these clinical strains. Comparing the present results with those obtained 3 years ago using gentamicin against 50 strains of the same baterial group, we noticed an evident loss in activity. We report the finding of a gentamicin-resistant tobramycin-sensitive *P. aeruginosa* strain; this resistance might be due to a chromosomic change rather than to an R-factor.

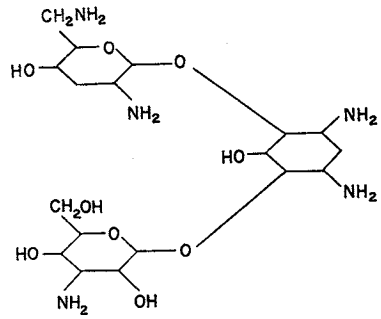
A complex of antibacterial compounds known as nebramycin[1,2,3,4] was first obtained from a *Streptomyces* strain from soil samples taken in Mexico, and now classified as a new species known as *Streptomyces tenebrarius* due to its extraordinary susceptibility to the light. The different compounds of the complex have been isolated chromatographically and purified. After microbiological studies, it was stated that factor 6, afterwards known as tobramycin[5], was the most active and showed a broader antimicrobial spectrum; it was found to be highly effective against *Pseudomonas* and *Proteus* among other bacteria.

The chemical structure of tobramycin (m. w. 467) is presented in Fig. 1, showing that the antibiotic is a member of the aminoglycoside group.[5] Due to the advantages in solubility tobramycin trihydrate is used.

In this report, we have included the results obtained *in vitro* using tobramycin trihydrate and gentamicin sulphate against 100 *P. aeruginosa* clinical strains and the 3 international standard strains mentioned below:

Escherichia coli N.C.T.C.	10,418 (control)
Pseudomonas aeruginosa N.C.T.C.	10,662***
Pseudomonas aeruginosa N.C.T.C.	10,701****

Fig. 1.



Tobramycin ($C_{18}H_{37}N_5O_9$)

* Communication presented at the 1st Symposium on Microbiology of Bacterial Infections, Sociedad Española de Microbiologia. Madrid, 1972.

** This report has been partially financed by the Ayuda de Investigacion No. 12-088-72 de la Comisión del Descuento Complementario del I.N.P.

*** Pyocinogenic capacity has been studied and typed as pyocinotype 31, according to GILLIES and GOVAN.[6,7,8]

**** Pyocinogenic capacity has been studied and found untypable according to GILLIES and GOVAN's technique.[6,7,8]

Material and Methods

A modified technique of the diffusion method was employed to determine the minimum inhibitory concentration (M.I.C.) of tobramycin and gentamicin against the 103 strains used during this study. Quantification was carried out by plotting to a correlation line using a standard strain. A report on this modification will be published shortly.

MUELLER-HINTON Agar OXOID (batch No. 119 12,234) was used as a medium according to the W.H.O. Antibiotic Committee[9,10] and M.J. WEINSTEIN[11] recommendations. The following sensitivity discs were used:

Gentamicin sulphate 30 mcg MAST (batch No. 7.419)
Tobramycin trihydrate 10 mcg B.B.L. (batch No. P-69, 808)

The disc concentrations have been controlled with discs elaborated by the authors using 740-E-Schleicher and Schuell Inc. paper (batch No. 5382), gentamicin sulphate (Schering Corp.) and tobramycim trihydrate (Ely Lilly of Spain). *E. coli* N.C.T.C. 10,418 was used as control for all determinations. All strains were previously isolated in enriched blood agar at 37°C, during a period of 24 hours.[12] Readings were taken 18 hours after incubation at 37°C.

Results

The MIC of the 3 standard strains are shown in Table 1. Both antibiotics show similar activity.

In Table 2, we can see the results of the 100 clinical *P. aeruginosa* strains* classified under MIC of each antibiotic. As it is shown, 1 mcg/ml tobramycin is capable of inhibiting 54% of the strains used and the same gentamicin concentration inhibits only 5 % of them. All the strains were inhibited by 4 mcg/ml or less of tobramycin except one which was inhibited by a higher concentration (8 mcg/ml). However, to obtain a similar effect, the gentamicin concentration was 4 times that of tobramycin (16 mcg/ml inhibits 99% of the strains). Four mcg/ml of gentamicin is active over 82% of these *P. aeruginosa* strains and tobramycin at the same concentration is active against 99%.

Table 1. *In vitro* activity of tobramycin and gentamicin against three international standard strains.

	MIC (mcg/ml)	
	Tobramycin	Gentamicin
E. coli N.C.T.C. 10,418	1	0.5
P. aeruginosa N.C.T.C. 10,662	2	4
P. aeruginosa N.C.T.C. 10,701	0.5	1

Fig. 2 shows graphically the same results as Table 2. Gentamicin MIC_{50} (MIC of 50 % of the sensitive strains) calculated in Fig. 2 is 2.4 mcg/ml. The tobramycin MIC_{50} is 0.9 mcg/ml. The relationship gentamicin MIC_{50}/tobramycin MIC_{50} is 2.6. These concentrations, 2.4 and 0.9

Table 2. Sensitivity of 100 *P. aeruginosa* strains

		MIC (mcg/ml)									
		0.5	1	2	4	8	16	32	64	128	>128
No. of strains	Tobramycin	6	48	33	12	1	—	—	—	—	—
	Gentamicin	1	4	36	41	13	4	—	—	—	1
Accumulative percentage	Tobramycin	6%	54%	87%	99%	100%	—	—	—	—	—
	Gentamicin	1%	5%	41%	82%	95%	99%	99%	99%	99%	100%

* 34 different pyocinotypes according to GILLIES and GOVAN's technique.[6,7,8]

mcg/ml are statistic figures and not standard test concentrations and must be corrected by the immediate superior ones. In MIC studies starting from 1 mcg/ml in progression to the basis of 2, 0.9 mcg/ml is represented as 1 mcg/ml and 2.4 mcg/ml as 4 mcg/ml. Therefore, the relationship expressed above should be 4/1=4, instead of 2.4/0.9=2.6.

As shown in Table 3, the MIC of 8 clinical *P. aeruginosa* strains is exactly the same for tobramycin and gentamicin. Nevertheless, 91 strains were inhibited by smaller concentrations of tobramycin, while only 1 strain was more sensitive to gentamicin. The isolation of a gentamicin-resistant and tobramycin-sensitive strain is, probably, the most interesting fact arising from this study. It was the *P. aruginosa* C.P.H. 10,527/72 pyocinotype 1 c,* isolated in two consecutive samples from an in-patient of our Clinic who had been previously treated with gentamicin. The MIC of tobramycin against this strain was 2 mcg/ml and >128 mcg /ml for gentamicin. We can clearly see in Fig. 3, that there is an inhibitory area surrounding the 10-mcg tobramycin disc (white disc) of 25 mm diametre, while the gentamicin disc (dark disc) containing the same concentration shows no inhibition at all.

We have initiated studies and are trying to locate the type of resistance and determine the properties of the possible enzyme responsible for inactivation. Up to now, we have been unable to

Fig. 2.

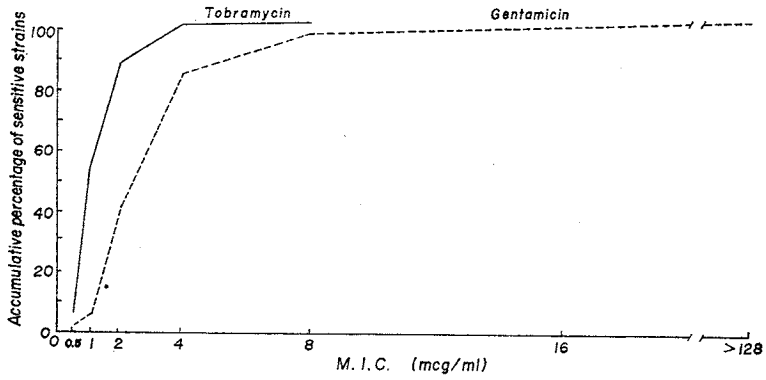
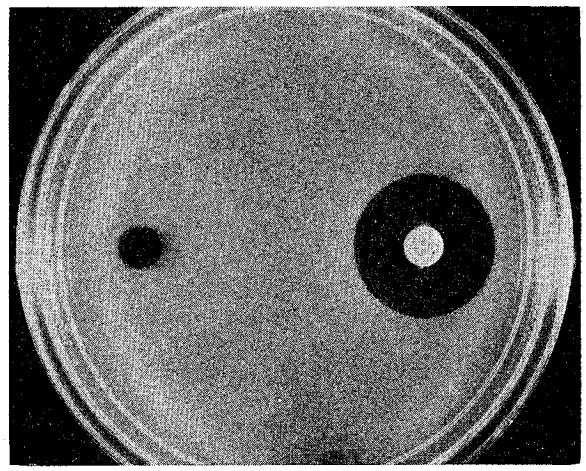


Table 3. *In vitro* activity of tobramycin and gentamicin against 100 clinical *Pseudomonas aeruginosa* strains

	No. of strains
Strains with the same MIC	8
Tobramycin MIC lower than gentamicin MIC	91
Gentamicin MIC lower than tobramycin MIC	1
Gentamicin-resistant-tobramycin-sensitive	1
Gentamicin-sensitive-tobramycin-resistant	0

Fig. 3. Sensitivity test of the *Pseudomonas aeruginosa* strain C.P.H. 10,527/72 to gentamicin (dark disc 10-mcg) and tobramycin (white disc 10-mcg).

The absence of inhibition round the gentamicin disc is clear, while there is a neat halo surrounding the tobramycin disc.



* GILLIES and GOVAN's technique.

transfer this resistance to *E. coli* K-12 E 711 F⁻, *E. coli* N. C.T.C. 10,418 nor *P. aeruginosa* N.C.T.C. 10,701, using the same method employed in our previous publication.[13]

Discussion

Tobramycin is a highly effective aminoglycoside antibiotic against gram-negative resistant strains such as *P. aeruginosa* and *Proteus*. The antibiotic has proved to be four times more active than gentamicin during a study of 100 *P. aeruginosa* clinical strains.

If we consider 4 mcg/ml as the highest MIC to classify strains as sensitive for the two antibiotics to treat systemic infections, the percentage of resistant strains is 1% for tobramycin and 18% for gentamicin. However, if we take into account that many of the strains were isolated from urine samples, in which we obtain, with average dosages, levels of 150~300 mcg/ml for tobramycin and 200~300 mcg/ml for gentamicin, the MIC limit should be 32 mcg/ml[14] and strains would be 100% sensitive to tobramycin and 99% to gentamicin. We isolated a gentamicin-resistant and tobramycin-sensitive strain with an MIC of >128 mcg/ml and 2 mcg/ml respectively. In 1969, we studied[15] the MIC of gentamicin against 50 *P. aeruginosa* clinical strains isolated during that year. The distribution into grounps according to the MIC in mcg/ml was as follows:

One strain of MIC 0.125; 4 strains of 0.25 MIC; 4 strains of 0.5 MIC; 26 strains of MIC 1; 11 strains of MIC 2; 2 strains of MIC 4; no strains of MIC 8; 1 strain of MIC 16 and 1 strain of MIC 32.

It can be seen that, three years ago, 70% of the strains were inhibited by 1 mcg/ml or less of gentamicin whereas this concentration would actually inhibit only 5% of the strains isolated recently in our laboratory; 96% of the strains studied in 1969 were sensitive to 4 mcg/ml or less of gentamicin, and actually only 82% of the present strains would be sensitive to this concentration. On that occasion, we were unable to detect strains completely resistant to gentamicin (the strain with the highest MIC 32 mcg/ml could be considered sensitive if isolated in urine specimen). At present, we have a strain needing >128 mcg/ml of gentamicin and would still remain resistant even when found in urine.

In 1971, MITSUHASHI *et al.*[16] described a gentamicin-resistant *P. aeruginosa* and demonstrated that antibiotic inactivation was obtained through a cell-free extract in presence of acetylcoenzyme A, although they did not determine the specificity of the enzyme nor the structure of the inactivated substance. In 1972, BRZEZINSKA *et al.*[17] published a report on clinical *P. aeruginosa* strains which were tobramycin-sensitive and gentamicin-resistant and proved that gentamicin inactivity took place by acetylation at 3-amino group of the 2-deoxystreptamine ring. This was the first time that the modification of the 2-deoxystreptamine ring had been described in an aminoglycoside antibiotic, therefore, it is a new type of enzymatic inactivation of these antibiotics. After analysis of extracts from the gentamicin-resistant *P. aeruginosa* strains, a new acetylating enzyme (gentamicin-acetyltransferase) with high specificity to gentamicin C, was discovered. Resistance was not cured through normal methods which do not allow the plasmids to replicate, nor transferred through usual conjugation techniques to sensitive strains, which brings us to the conclusion that the gene or genes responsible for the production of this acetylating enzyme must not be extrachromosomal. From early studies of the gentamicin-resistant strain isolated in our laboratory, we think that it could carry a similar type of resistance. Up to now, we have no evidence for an extracellular transferable substance involved in inactivation of tobramycin.

The results published in the present study compared to those obtained in 1969 concerning gentamicin activity against clinical *P. aeruginosa* strains suggest a chromosomal resistance in multiple steps, and although bacteria are still sensitive to this drug we notice a gradual loss in activity. Other authors[18] are of a similar opinion, although we must bear in mind that *P. aeruginosa* strains have been reported with extrachromosomal transferable resistance to gentamicin.[15]

Nevertheless, gentamicin is still a leading antibiotic in *P. aeruginosa* infections, although in cases where resistant or intermediate strains appear, tobramycin may remain sensitive. In our opinion this dissociation, though it takes plate only in few cases, would justify the acceptance of

tobramycin as a commercial drug.

Activity studies of both aminoglycosides on standard strains, show that tobramycin is slightly more active in the two *Pseudomonas* strains used and slightly less active in the *E. coli* strain studied. We think that gentamicin lower activity over clinical *P. aeruginosa* strains is due to the prolonged employment as a leading antibiotic while tobramycin is a new member of this group. After several years of commercialization, tobramycin will probably decrease in activity too; there are already reports of tobramycin-resistant strains and that the resistance has been transferred to sensitive strains.[19]

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