ISOLATION OF *S. TYPHI* RESISTANT TO HIGH CONCENTRATIONS OF CHLORAMPHENICOL[1]

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There is clear evidence that Mexico's 1972 typhoid fever epidemic was caused by a drug-resistant strain of Salmonella typhi *that intermingled with endemic strains. The resistance factors involved bear some similarity to factors found in strains of* Shigella dysenteriae *1 implicated in the recent dysentery epidemic affecting Central America and parts of Mexico.*

Introduction

Until now, chloramphenicol has been considered the drug of choice for treating typhoid fever. Nevertheless, several published articles have reported finding strains of *Salmonella typhi* resistant to high concentrations of chloramphenicol in various countries of Asia and Africa. The percentage of resistant strains is said to have run as high as 20 per cent in India and 25 per cent in Nigeria (*1-5*). Before 1972, as far as is known, all isolated cultures of *S. typhi* from Mexico had invariably proven sensitive to this antibiotic (*6-8*).

In February 1972 a major typhoid fever epidemic broke out in Mexico City and in Pachuca, Hidalgo State, which later spread to other areas. Incomplete reports indicate there were over 2,000 cases in the Federal District and Hidalgo State alone between February and May of that year (*9*).

From the start of the epidemic, great importance was attributed to the isolation of *S. typhi* resistant to chloramphenicol. The existence of such strains was reported sumultaneously by several hospitals in Mexico City, strongly suggesting that the epidemic was being caused primarily by a chloramphenicol-resistant strain. This evidence was supported by the fact that a great majority of the isolated bacterial cultures, besides showing resistance to chloramphenicol, belonged to a peculiar phage-type identified as "degraded Vi approaching phage-type A." Further confirmation was provided by the clinical observation that chloramphenicol treatment was apparently unsuccessful in a significant percentage of patients. This latter development will be dealt with in a separate study.

Materials and Methods

A total of 486 *S. typhi* cultures were tested using the disk method. These had been isolated in the laboratories of the Hospital for Infectious Diseases of the La Raza Medical Center (Mexican Social Security Institute), the Institute of Public Health and Tropical Diseases, the Children's Hospital of Mexico, and the Pediatric Hospital of the Mexican Social Security Institute.

From among these cultures, 327 collected in a variety of localities (see Table 1) were selected at random for chloramphenicol sensi-

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TABLE 1–Resistance to 50 μg/ml of chloramphenicol by 327 *S. typhi* strains isolated in various parts of Mexico between March and July 1972.

Area	No. of strains		Percentage of resistant strains
	Tested	Resistant to 50 μg/ml	
Mexico City and neighboring areas of the State of Mexico	226	214	94.7
Pachuca, State of Hidalgo	28	26	92.9
Tlaxcala, State of Tlaxcala	11	10	90.9
Puebla, State of Puebla	28	22	78.6
Zacatecas, State of Zacatecas	12	10	85.0
San Luis Potosí, State of S.L.P	18	18	100.0
Poza Rica, State of Veracruz	4	3	75.0
Total	327	303	92.7

tivity tests employing the Jackson and Finland plate dilution method (*10*). In addition, a group of 100 *S. typhi* cultures selected at random were tested by this method for resistance to 10 antimicrobial agents (chloramphenicol, tetracycline, streptomycin, ampicillin, cephalothin, colistin, gentamicin, kanamycin, nalidixic acid, and nitrofurantoin–see Table 2). In both of these experiments the following drug concentrations were tested: 0.6, 1.25, 2.5, 5, 10, 20, 50, 100, 200, 300, 400, and 500 μg/ml.

In addition, the phage-types of 49 of the *S. typhi* cultures were determined at the Center for Disease Control in Atlanta, Georgia, courtesy of Dr. George J. Hermann.

TABLE 2–Sensitivity of 100 *S. typhi* strains to 10 antimicrobial agents.

Antimicrobial agent	No. of strains	
	Sensitive to 5.0 μg/ml or less	Resistant to 200 μg/ml or more
Chloramphenicol	12	88
Tetracycline	12	88
Streptomycin	12	88
Ampicillin	100	0
Cephalothin	100	0
Colistin	100	0
Gentamicin	100	0
Kanamycin	100	0
Nitrofurantoin	100	0
Nalidixic Acid	100	0

Results and Conclusions

Of 486 *S. typhi* cultures tested, 445 (92 per cent) resisted over 50 μg/ml of chloramphenicol. As shown in Table 1, the proportion of resistant strains among the 327 selected cultures varied from 75 to 100 per cent in different areas, the average being 92.7 per cent. All of the 100 *S. typhi* cultures tested for sensitivity to other antimicrobial agents proved uniformly sensitive to ampicillin, cephalothin, colistin, gentamicin, kanamycin, nitrofurantoin, and nalidixic acid at concentrations varying from 0.6 to 5 μg/ml. However, 88 of these cultures resisted over 200 μg/ml. of chloramphenicol, tetracycline, and streptomycin. The 12 sensitive cultures were inhibited by these three antibiotics at concentrations of less than 5 μg/ml.

Table 3 shows the phage-type distribution of 49 *S. typhi* cultures grouped according to their areas of origin and their sensitivity or resistance to chloramphenicol. All strains termed "resistant" grew at concentrations of 200 μg/ml or more, while all "sensitive" strains were inhibited by 5 μg/ml or less. It was found that all 38 resistant strains belonged to the phage-type "degraded Vi approaching phage-type A"; however, 9 of the 11 sensitive strains were of phage-type E-1, one was of phage-type 26, and only one belonged to "degraded Vi approaching

TABLE 3–Phage-types of 49 *S. typhi* strains isolated in different parts of Mexico.

Area	No. of strains tested	Phage-type of strains tested					
		E-1		26		Degraded Vi approaching phage-type A	
		S[a]	R[b]	S	R	S	R
Mexico City and neighboring areas of the State of Mexico	20	6	–	1	–	1	12
Tlaxcala, State of Tlaxcala	12	–	–	–	–	–	12
Hidalgo State	9	–	–	–	–	–	9
Puebla, State of Puebla	8	3	–	–	–	–	5
Total	49	9	–	1	–	1	38

[a] Sensitive.
[b] Resistant.

phage-type A." It is interesting to recall that all three of these phage-types have been found in Mexico for over 15 years (*11*).

Unpublished studies by Drs. J. Olarte and E. Galindo, as well as work carried out at the CDC in Atlanta, have shown that the resistance to chloramphenicol, tetracycline, and streptomycin of the *S. typhi* cultures isolated during the epidemic depends on certain episomes or resistance (R) factors; these appear identical to the episomes found in the strains of *Shigella dysenteriae* 1 responsible for the recent dysentery epidemic in Central America and parts of Mexico, including Hidalgo State and the Federal District (*12, 13*). Similar episomes were discovered earlier in Mexico among *Shigella flexneri* and other enteropathogenic bacteria (*14*). The fact that the episomes of the epidemic strains of *S. dysenteriae* 1 and *S. typhi* could have had a common origin involves important implications; for besides conferring resistance to antibiotics, such episomes might have endowed these bacterial strains with a virulence responsible for their extreme contagiousness and their apparent ability to provoke particularly severe clinical manifestations (*15*). Though this point will be the subject of a future article, it is important to stress that *S. typhi* resistance to chloramphenicol has had a considerable impact on treatment, obliging hospitals to employ other apparently less effective drugs.

Addendum

Recent information from Dr. David H. Smith of the Children's Hospital Medical Center in Boston, Massachusetts, indicates there are important genetic differences between the R factors found in epidemic *S. dysenteriae* 1 from Central America and epidemic *S. typhi* from Mexico. A summary of this data appears in "Abstracts of the Annual Meeting of the American Society for Microbiology, 1973."

SUMMARY

A high proportion (92 per cent) of *S. typhi* cultures isolated during Mexico's 1972 typhoid fever epidemic showed strong resistance to chloramphenicol. All the cultures resistant to chloramphenicol were equally resistant to tetracycline and streptomycin, but were sensitive to seven other antimicrobial agents, including ampicillin.

Of the cultures sensitive to chloramphenicol, only one belonged to phage-type "degraded Vi approaching phage-type A." On the other hand, all the resistant cultures for which the phage-

type was determined belonged to "degraded Vi approaching phage-type A." These findings confirm indications that the epidemic was caused by a single strain with multiple drug-resistance, intermingled with endemic strains. Eight per cent of the *S. typhi* cultures examined were sensitive to chloramphenicol; all of these, with a single exception, belonged to other phage types (E-1 and 26).

The similarity between the multiple drug-resistance of this epidemic *S. typhi* strain and that of the *Shigella dysenteriae* 1 strain responsible for the recent dysentery epidemic in Central America and Mexico raises the possibility that the two episomes had a common origin.

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