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Salmonella typhi resistant to Chloramphenicol, Ampicillin, and Other Antimicrobial Agents: Strains Isolated During an Extensive Typhoid Fever Epidemic in Mexico

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During 1972 a large epidemic, in excess of 10,000 cases, of typhoid fever occurred in Mexico City, Pachuca, and other communities of Mexico. The main characteristic of the epidemic, in addition to the large number of persons affected, was the prevalence of a strain of *Salmonella typhi* which was highly resistant to chloramphenicol both in vivo and in vitro, and which belonged to a single phage type, Vi degraded approaching type A. Of 493 strains of *S. typhi* studied during the outbreak, 452 (91.7%) were resistant to chloramphenicol (CM), tetracycline (TC), streptomycin (SM), and sulfonamides (SU). The epidemic strain owes its resistance to an R factor which is easily transferable to *Escherichia coli* K-12 and which appears to be stable. In the third month of the outbreak, a strain of *S. typhi* resistant to CM, TC, SM, SU, ampicillin (AM), and kanamycin (KM) was isolated from a patient with severe typhoid fever. During the following 9 months, six additional strains of *S. typhi* resistant to AM, CM, TC, SM, and SU were also isolated. Transfer experiments to *E. coli* K-12 indicate that these strains are infected with two different R factors, one causing CM, TC, SM, and SU resistance and the other causing AM or AM and KM resistance. The frequency of transfer of the resistance in overnight crosses was in the order of 10^{-4} for CM, TC, SM, and SU and 10^{-6} for AM or AM, and KM. The appearance of these strains resistant both to chloramphenicol and ampicillin was a cause for concern for the clinicians; fortunately, they have remained an infrequent cause of disease.

In February 1972, an epidemic of typhoid fever began explosively in Mexico City and Pachuca (State of Hidalgo), in subsequent months spreading to several other communities in Mexico including Tulancingo, Apizaco, Puebla, Poza Rica, and Acapulco. The incidence of the disease was highest during March and April; however, a recrudescence was observed in Mexico City between July and October. Sporadic cases in unusually high numbers have been reported through the end of 1972 and the beginning of 1973. Information not yet complete indicates that during 1972 more than 10,000 confirmed cases of typhoid fever were observed. The age groups most commonly affected were children between 5 and 14 years of age and young people between the ages of 15 and 24 years.

The main characteristic of the epidemic, in addition to its rapid spread and the large number of persons affected, particularly in the cities of Mexico, Pachuca, and Tulancingo, was the prevalence of a strain of *Salmonella typhi* highly resistant to chloramphenicol both in vivo and in vitro. During the first 6 months of the outbreak, over 400 strains of *S. typhi* were recovered in seven different communities. Of these isolates, about 92% were multiply resistant to chloramphenicol (CM), tetracycline (TC), streptomycin (SM), and sulfonamides (SU), but susceptible to ampicillin (AM), cephalothin (CEP), colistin (COL), gentamicin (GT), nitrofurantoin (NIT), and nalidixic acid (NAC) (3). In the course of the epidemic, seven strains of *S. typhi* were isolated which were resistant to CM, TC, SM, and SU, and which also grew in high concentrations of AM. One of these strains was also resistant to

kanamycin. The purpose of this paper is to present information regarding the nature of the resistance of the cultures of *S. typhi* isolated during the epidemic.

MATERIALS AND METHODS

Strains studied. Between March 1972 and July 1972, a total of 493 *S. typhi* strains were isolated by the laboratories of the Hospital de Infectologia del Centro Medico La Raza (IMSS) (P. Mendoza-Hernandez), Instituto de Salubridad y Enfermedades Tropicales (D. Bessudo), Hospital de Pediatria (IMSS) (G. Gutiérrez-Trujillo), and Hospital Infantil de México. A majority of the strains were cultured from patients with a clinical syndrome compatible with typhoid fever.

Antimicrobial drugs tested. Freshly prepared solutions of the following drugs were used: CM, TC, SM, AM, CEP, COL, GT, KM, NIT, NAC, and SU.

The following Sensi-discs (BBL) were also used (micrograms): CM, 30; TC, 30; SM, 10; AM, 25; CEP, 30; COL, 10; GT, 10; KM, 30; NIT, 100; NAC, 30; and SU, 1,000.

Test for sensitivity. The plate dilution method described elsewhere (8) was used throughout the study. Brain heart-infusion agar (1.75%, BBL), plus the respective antimicrobial drug, was used. The final volume was 10 ml of medium per plate. The final drug concentrations were 0.6, 1.25, 2.5, 5, 10, 20, 50, 100, 200, 300, 400, and 500 μg/ml. When indicated, higher concentrations were also used.

Each agar plate was inoculated with a 3-h broth culture by using sterile swabs for streaking. This seed culture was prepared by inoculating 10 ml of broth with 0.1 ml of a 20-h broth culture. The end point was taken as the minimal antibiotic concentration producing inhibition for 20 h when incubated at 37 C (MIC).

When indicated, the single-disk technique described by Bauer et al. (2) was employed, with Trypticase soy agar (BBL) or Mueller-Hinton agar for sulfonamides testing.

Transfer of drug resistance. Single colonies of resistant *S. typhi* and multiply sensitive *Escherichia coli* K-12 strain W-1485 were inoculated separately in 20 ml of brain heart infusion broth and incubated at 37 C for 16 to 18 h. After adjusting the optical density of the cultures to give a concentration of about 10^8 bacteria per ml, 9 ml of the donor strains was mixed with 1 ml of *E. coli* K-12 receptor strains. The mixed cultures were incubated under the same conditions as above, diluted 1:10 in fresh broth, and incubated again at 37 C for 90 min. A 0.1-ml volume of 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-7} dilutions of the mixtures in sterile saline were seeded onto plates of MacConkey agar containing the appropriate, single, selecting drug. After 24 to 48 h of incubation at 37 C, the plates were inspected for colonies of resistant *E. coli*. From three to six colonies were picked, purified by plating on ordinary MacConkey agar, confirmed as *E. coli* by biochemical tests, and tested for the pattern of drug resistance by the disk method. When indicated, the level (MIC) of transferred resistance was measured by the plate dilution technique.

Normally, the drug concentrations used in the selection medium were 25 μg of CM and TC per ml and 10 μg of SM per ml, except where indicated otherwise.

RESULTS

Of 493 strains of *S. typhi* tested, 452 (91.7%) were resistant to CM at concentrations of 50 μg/ml or higher.

The resistance pattern of 101 strains of *S. typhi* to 11 drugs was determined by using the plate dilution method. All 101 strains tested were inhibited by 0.6 to 5 μg of CEP, COL, GT, NIT, and NAC per ml. Eighty-nine strains were simultaneously resistant to 100 to 300 μg of CM per ml, 100 to 200 μg of TC per ml, 50 to 200 μg of SM per ml, and more than 1,000 μg of SU per ml. One strain in addition to being multiply resistant to CM, TC, SM, and SU, grew in 1,000 μg of KM per ml and 5,000 μg of AM per ml.

Ten strains of *S. typhi* with multiple resistance to CM, TC, SM, and SU were tested for their ability to transfer the resistance to *E. coli* K-12 strain W1485). In a single experiment (Table 1), 7 out of 10 strains proved capable of simultaneously transferring the resistance determinants for the four drugs. In three strains, resistance only to TC was transferred to *E. coli*. The transfer frequency of the resistance from *S. typhi* to *E. coli* was in the order of 10^{-4} in overnight crosses.

Two strains of *E. coli* K-12 which received the resistance factors CM, TC, SM, and SU from *S. typhi* were also tested for their ability to transfer the experimentally acquired resistance to two multiply sensitive strains of *S. typhi*, one isolated before the outbreak (strain HI-1969) and one isolated during the outbreak (strain 34271-1972). Resistance to the four drugs was transferred back from *E. coli* to both sensitive strains of *S. typhi*. In this case, the frequency of

TABLE 1. *Transferability of drug resistance from Salmonella typhi (typhoid fever outbreak: Mexico, 1972) to a strain of Escherichia coli*

S. typhi donor strains resistant to CM, TC, SM, and SU	Resistance transferred to *E. coli* K-12[a]
H-1	TC
H-14	CM, TC, SM, SU
H-27	CM, TC, SM, SU
H-30	CM, TC, SM, SU
H-72	CM, TC, SM, SU
H-78	CM, TC, SM, SU
H-JRS	TC
H-VC	CM, TC, SM, SU
H-FU	CM, TC, SM, SU
C-5	TC

[a] Multiply sensitive strain W1485.

transfer of the resistance was in the order of 10^{-6} in overnight crosses.

In the course of the outbreak between May and September, seven strains of *S. typhi* were isolated which were resistant to CM, TC, SM, and SU, but at the same time which were resistant to more than 5,000 μg (highest concentration used) of AM per ml. One of these strains (strain H-185) was also resistant to KM (1,000 μg/ml). Table 2 shows the origin and date of isolation of these seven strains, together with the levels of resistance to 10 different drugs.

Transfer experiments to *E. coli* K-12 from *S. typhi* strains resistant to AM, CM, TC, SM, and SU, using various selecting drugs and examining only one recipient colony from each selecting drug, indicate that in five out of seven strains tested the resistance to AM alone was transferred when this antibiotic was used in the selective plates (Table 3). In contrast, when CM or TC was used as the selecting antibiotic, in six out of seven strains the resistance transferred was that to CM, TC, SM, and SU but not to AM. In the case of strain H-185 (which in addition to resistance to AM, CM, TC, SM, and SU was also resistant to KM), on AM and KM selective plates resistance was transferred to AM and KM but not to CM, TC, SM, and SU; in TC selective plates resistance transfer was observed to CM, TC, SM, and SU but not to AM and KM. The frequency of transfer of the resistance in overnight crosses was in the order of 10^{-4} for CM, TC, SM, and SU and 10^{-6} for AM or AM and KM.

DISCUSSION

For many years, CM has been considered the treatment of choice for typhoid fever. Until 1972 there was no known record of the existence in Mexico of *S. typhi* resistant to CM (12, 14, 16, 18) despite the extensive use of this antibiotic throughout the country and the increasingly frequent appearance since 1955 of strains of *Shigella*, *Salmonella* of animal origin, and enteropathogenic *Escherichia coli*, all resistant to multiple drugs including CM (13, 15, 19). The resistance of a large proportion of these enteric bacteria isolated in Mexico has been shown to be due to transferable R factors (5, 15, 19). To our knowledge, the epidemic of typhoid fever observed in Mexico in 1972 is the first outbreak of the disease produced by *S. typhi* resistant to CM. However, strains of *S. typhi* resistant to this and other antimicrobial drugs have been isolated previously from sporadic cases of typhoid fever in India, West Africa, Greece, Israel, Spain, and Chile (1, 4, 9–11). The subject of drug-resistant *S. typhi* was recently summarized by Anderson and Smith (1).

Typhoid fever has been endemic in Mexico for many years, and outbreaks have occurred from time to time. However, an epidemic of such magnitude has not been observed in this country during the last 30 years. On the contrary, at least in Mexico City, the incidence of typhoid fever has progressively declined during the previous 20 or 25 years, in large part due to adequate chlorination of the water supply and modernization of the city's public markets.

The explosive onset of the outbreak suggested a common source of the infection with massive distribution of the typhoid bacillus such as through water or food; unfortunately, studies carried out by public health authorities failed to detect either the source of the epidemic or the vehicle of transmission. Considering the large

TABLE 2. *Resistance levels to multiple drugs, of seven unusual strains of Salmonella typhi isolated during the outbreak of typhoid fever observed in Mexico, 1972*

Strain	Date of isolation and locality	Sulfadiazine[a] disk technique	Minimal inhibitory concn (μg/ml)[b]								
			AM	CM	TC	SM	KM	CEP	COL	GT	NAC
H-185, blood	30/5/72 Mexico City	Resistant	>5,000	300	200	200	1,000	10	1.25	5	5
La Raza 1, bone marrow	8/6/72 Mexico City	Resistant	>5,000	300	200	200	5	10	1.25	5	5
La Raza 2, bone marrow	24/6/72 Mexico City	Resistant	>5,000	300	200	200	5	10	1.25	5	5
Puebla 12, feces	5/8-72 Puebla, Pue.	Resistant	>5,000	300	200	300	5	10	1.25	5	5
JRR, feces	25/9/72 Acapulco, Gro.	Resistant	>5,000	300	200	200	5	10	1.25	5	5
LA, feces	22/9/72 Mexico City	Resistant	>5,000	300	200	200	5	10	2.5	5	5
JM, blood	28/11/72 Tulancingo, Hgo.	Resistant	>5,000	300	200	200	5	10	2.5	5	5

[a] Sulfadiazine—1,000 μg.
[b] Plate dilution.

TABLE 3. *Levels of drug resistance received by Escherichia coli K-12 (multiply sensitive strain W1485) from seven strains of Salmonella typhi resistant to multiple drugs, isolated during the typhoid fever outbreak observed in Mexico, 1972*

S. typhi donor strains[a]	Selecting drug (μg/ml)	Minimal inhibitory concn (μg/ml) of recipient colonies of *E. coli*[b]					Sulfadiazine disk technique[c]
		AM	KM	CM	TC	SM	
H-185	AM (200)	>5,000	500	10	2.5	10	S
	TC (25)	10	2.5	300	200	50	R
	KM (100)	4,000	1,000	5	5	10	S
La Raza 1	AM (200)	>5,000		10	2.5	10	S
	CM (200)	5	5	300	200	25	R
La Raza 2	AM (200)	>5,000	5	10	2.5		S
	CM (200)	5	5	300	200	25	R
Puebla 12	AM (200)	>5,000	10	25	0.6	300	S
	CM (200)	5	10	300	300	200	R
JRR	AM (200)	>5,000	10	300	200	100	R
	CM (200)	5	10	300	200	200	R
LA	AM (25)	>5,000	10	300	200	300	R
	CM (25)	100	10	300	200	100	R
JM	AM (25)	3,000	10	5	50	200	R
	CM (25)	2.5	10	300	200	100	R

[a] See original levels of resistance of these strains in Table 2.
[b] Only one colony tested from each selecting drug.
[c] R, Resistant; S, susceptible.

number of active cases and of carriers, there can be no doubt that person-to-person transmission also played an important role in the spread of the disease.

The very high proportion (91.9%) of CM-, TC-, SM-, and SU-resistant strains of *S. typhi* recovered during the epidemic, together with the fact that 148 (97%) out of 153 resistant strains which were phage-typed at the Center for Disease Control (Atlanta, Ga., George J. Hermann) (3) and the Pasteur Institute (Paris, J. F. Vieu) were of the same phage type (Vi degraded approaching type A) (the other resistant strains were W forms), suggests that the epidemic of typhoid fever was caused by a single strain, with the occasional occurrence of cases due to sensitive endemic strains.

The epidemic strain owes its resistance to an R factor transferable to *E. coli* K-12, as was shown in this study as well as in experiments carried out in the United States (7) and England (1). The R factor originating from *S. typhi* is also easily transferable from *E. coli* K-12 to multiply sensitive strains of *S. typhi* and appears to be stable.

The R factor from epidemic *S. typhi* confers the same pattern of resistance as the R factor found in pandemic strains of *Shigella dysenteriae* 1 (CM, TC, SM, and SU) isolated in Central America in recent years (6, 7, 19), and which were also present in Mexico City and Pachuca at the beginning of the typhoid outbreak. However, according to studies carried out by T. L. Marsh and D. H. Smith (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 63, 1973), there are considerable genetic differences between the two R factors which suggest different origins. In any case, these two epidemics are good examples of the potential dangers produced by the infection of pathogenic bacteria of high virulence, like *S. typhi* or *S. dysenteriae* 1, with R factors, particularly in countries with poor sanitation.

At the end of the third month of the outbreak, a strain of *S. typhi* resistant to CM, TC, SM, and SU but also resistant to AM and KM was isolated from a patient with severe typhoid fever. Since AM was considered by the clinicians as the drug of choice for the treatment of disease due to the CM-resistant strains, the possibility that the epidemic strain of *S. typhi* had acquired this new resistance was a serious cause for concern. Over the next few months, particular emphasis was placed on the detection

of cultures of *S. typhi* resistant to AM and CM. Fortunately, during the following 9 months only six additional strains with these characteristics were found among several hundred cases of typhoid fever studied; four strains were isolated in Mexico City and one each from the cities of Puebla, Acapulco, and Tulancingo, and at widely separate times. Furthermore, preliminary phage-typing data indicate that only two strains (Puebla 12 and JM) are of the same type as the epidemic strain of *S. typhi*. Strain JRR is a W form, and the other four strains are Vi degraded with various phage patterns (George J. Hermann, Center for Disease Control, Atlanta). However, the levels of resistance demonstrated by these seven strains to CM, TC, SM, and SU are similar to those of the epidemic strain of *S. typhi*. Transfer experiments of the resistance found in these strains to sensitive *E. coli* K-12 suggest the presence of two different R factors, one causing CM, TC, SM, and SU resistance, the other causing AM or AM and KM resistance, at different frequencies. It is not known whether these were endemic strains which, by chance, became infected both with the R factor present in the epidemic strain and with the R factor causing resistance to AM or AM and KM, or whether the epidemic strain became infected with a new plasmid which at the same time caused profound changes in the phage pattern and a diminished capacity for transmission. The latter could explain the apparent low incidence of the disease due to these strains during the course of the outbreak. Detailed studies of the nature of the genetic material present in these strains are in progress and will be the subject of a future publication.

The possibility that the epidemic strain of *S. typhi* possesses, in addition to the R factor (CM, TC, SM, and SU), an enhanced virulence or a factor which facilitates its transmissibility has been the subject of much speculation recently and remains to be resolved.

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LITERATURE CITED

1. Anderson, E. S., and H. R. Smith. 1972. Chloramphenicol resistance in the typhoid bacillus. Brit. Med. J. **3**:329-331.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. **45**:493-496.
3. Bessudo, D. M., J. Olarte, P. Mendoza-Hernandez, E. Galindo, J. Carrillo, G. Gutierrez-Trujillo, and J. Kumate. 1973. Aislamiento de *S. typhi* resistance a altas concentraciones de cloranfenicol. Bol. Ofic. Sanit. Panamer. **64**:1-6.
4. Chandra, R. K., R. Manchanda, O. N. Bahkoo, and K. C. Aggarwall. 1965. Chloramphenicol resistant enteric fever with hematological complications in a child. Indian J. Pediat. **32**:313-315.
5. Evans, J., E. Galindo, J. Olarte, and S. Falkow. 1968. Beta-lactamase of R factors. J. Bacteriol. **96**:1441-1442.
6. Farrar, W. E., and M. Edison. 1971. R factors in strains of *Shigella dysenteriae* type 1 isolated in the Western Hemisphere during 1969-1970. J. Infect. Dis. **124**:327-329.
7. Gangarosa, E. J., J. V. Bennett, C. Wyatt, P. E. Pierce, J. Olarte, P. Mendoza-Hernandez, V. Vázquez, and D. M. Bessudo. 1972. An epidemic-associated episome? J. Infect. Dis. **126**:215-218.
8. Jackson, G. G., and M. Finland. 1951. Comparison of methods for determining sensitivity of bacteria to antibiotics in vitro. Amer. Med. Ass. Arch. Intern. Med. **88**:446-460.
9. Murti, B. R., K. Rajvalakshmi, and C. S. Bhaskaran. 1962. Resistance of *Salmonella typhi* to chloramphenicol. J. Clin. Pathol. **15**:544-554.
10. Nare, I. J. 1967. Drug resistance in *Salmonella typhosa*. South. Afr. Med. J. **41**:703-704.
11. Nijku-Obi, A. N., and J. C. Njoku-Obi. 1965. Resistance of *Salmonella typhosa* to chloramphenicol. J. Bacteriol. **90**:552-553.
12. Olarte, J. 1969. Quimioterapia de las Salmonelosis. Gac. Med. Mex. **99**:642-648.
13. Olarte, J., and J. A. de la Torre. 1959. Resistance of *Shigella flexneri* to tetracyclines, chloramphenicol and streptomycin. A study of 131 freshly isolated strains. Amer. J. Trop. Med. Hyg. **8**:324-326.
14. Olarte, J., and G. Figueredo. 1955. The sensitivity of *Salmonella typhi* to synnematin B and other antibiotics. A study of forty freshly isolated strains. Antibiot. Chemother. **5**:162-165.
15. Olarte, J., and E. Galindo. 1970. Factores de resistencia a los antibioticos encontrados en bacterias enteropatogenas en la Ciudad de México. Rev. Lat. Amer. Microbiol. **12**:173-179.
16. Olarte, J., E. Galindo, and A. Joachin. 1963. Sensitivity of *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* to cephalothin, ampicillin, chloramphenicol, and tetracycline. Antimicrob. Ag. Chemother. 1962. p. 787-793.
17. Olarte, J., E. Galindo, and A. Joachin. 1965. El problema de la resistencia de las bacterias enteropatógenas a los antibioticos de uso común, p. 225-232. *In* III Simposio Panamericano de Farmacología y Terapéutica. Excerpta Med. Int. Congr. Ser. 127.
18. Olarte, J., and O. Mayes. 1957. La sensibilidad de *S. typhi*, *S. typhimurium*, *Sh. Flexneri*, y *E. coli* (enteropatógena), al cloranfenicol, a la tetraciclina y a la combinación de estos dos antibioticos. Bol Med. Hosp. Infant. Mex. **14**:589-596.
19. Olarte, J., G. Varela, and E. Galindo. 1971. Infeccion por *Shigella dysenteriae* 1 (Bacilo de Shiga) en Mexico. Bol. Med. Hosp. Infant. Mex. **28**:605-612.