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Resistance of *Salmonella typhosa* to Chloramphenicol

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Reports on the resistance pattern of salmonellae to chloramphenicol appear to be in conflict. Colquhoun and Weeth (Lancet **2**:621, 1950) reported increased resistance of *Salmonella typhosa* to chloramphenicol, in a case of relapsed typhoid fever. On the other hand, Good and Mackenzie (Lancet **1**:611, 1950) and Rankin and Grimble (Lancet **1**:650, 1950) did not find any such increase in similar cases. McWhorter et al. (Appl. Microbiol. **11**:368, 1963) found only 1 of 652 *Salmonella* cultures isolated in the United States in 1962 to be resistant to chloramphenicol. Also 1 of 335 *Salmonella* cultures isolated in Canada in 1962 was found to be resistant to chloramphenicol (Yurack, Can. J. Microbiol. **10**:521, 1964). In the Netherlands, Manten et al. (Tijdschr. Diergeneesk. **88**:1858, 1963) reported 10.94% of *Salmonella* cultures isolated in 1962 to be resistant to tetracycline, chloramphenicol, or both. Of this 10.94%, 3.4% were resistant to chloramphenicol. A much higher percentage (approximately 20%) of *S. typhosa* isolated in India was reported by Murti et al. (J. Clin. Pathol. **15**:544, 1962) to be resistant to high concentrations of chloramphenicol.

Our findings tend to support the observation that high resistance of salmonellae, particularly *S. typhosa*, to chloramphenicol may not be uncommon. Of 31 strains of *S. typhosa* isolated here in 1963, 25% were resistant to chloramphenicol when tested with Multidisk (Oxoid) no. 11-14C containing 10 μg of chloramphenicol. Quantitation of the resistance to chloramphenicol of the isolates, by the pour-plate method, showed that it varied from 15 to 250 μg/ml among the resistant strains.

The origin of such high resistance among the *S. typhosa* isolates could not, from the evidence available, be determined with certainty. It could not be definitely established that the patients from whom the organisms were isolated were previously on the antibiotic. This cannot, however, be excluded, since antibiotics and drugs are freely available here without prescription or medical supervision. They are on sale to the general public not only in pharmacies but also in many of the market stalls (Collard, W. African Med. J. **8**:197, 1959).

It is, therefore, possible that the apparent disagreement in reports on the resistance of salmonellae to chloramphenicol might reflect differing practices in the usage and control of the antibiotic in various geographical locations. It is also possible that such high resistance could have resulted from episome transfer from *Escherichia coli* resistant to chloramphenicol (Watanabe, Bacteriol. Rev. **27**:87, 1963). A very high percentage of *E. coli* strains (67% of 52 strains) isolated from patients here exhibited high resistance to the antibiotic (*unpublished data*).

A possible contribution to the mechanism of resistance to chloramphenicol was observed. Homogenates from three of the *S. typhosa* isolates which showed high resistance to chloramphenicol (125 to 250 μg/ml) inhibited the action of chloramphenicol on susceptible strains of *S.*

TABLE 1. *Effect of the homogenate of a chloramphenicol-resistant Salmonella typhosa on the action of chloramphenicol on susceptible bacteria**

Treatment	Organisms			
	S. typhosa B5	*Escherichia coli* 112	*E. coli* 570	*Staphylococcus aureus* (Oxford strain)
Chloramphenicol + phosphate-buffered saline (*p*H 7.2)	2.5	2.5	1.25	0.625
Chloramphenicol† + homogenate	125	50	75	65
Tryptone Soya Broth + homogenate	Confl	Confl	Confl	Confl

* Results expressed as the minimal inhibitory concentration of chloramphenicol in micrograms per milliliter. Confl = confluent growth.

† Sterile homogenate was added to various concentrations of chloramphenicol in 3 ml of Tryptone Soya Broth (Oxoid). After incubation at 37 C for 1 hr, 0.1 ml of an 18-hr broth culture of the organisms was added, and the test system was further incubated at 37 C for 18 to 24 hr. Blood-agar plates were streaked to check for growth or sterility.

typhosa, *E. coli*, and an Oxford strain of *Staphylococcus aureus* (Table 1).

The homogenate was obtained in the following manner. The organisms were grown on Tryptone Soya Agar Medium (1.5% of Oxoid Agar No. 3 was added to Oxoid Tryptone Soya Broth) in Roux bottles. After incubation at 37 C for 18 hr, the bacterial cells were harvested and washed three times with phosphate-buffered saline (PBS), *p*H 7.2. The packed cells, suspended in a small volume of PBS, were disintegrated with a Mickle disintegrator at 4 C.

The bacterial liquor was then centrifuged in a Sorvall refrigerated automatic centrifuge at 4,500 × *g* for 30 min at 0 C. The supernatant fluid was filtered through a sintered-glass filter and checked for sterility by plating on blood-agar medium.

Chloramphenicolase was reported in broth culture filtrates of chloramphenicol-resistant *S. typhosa* by Murti et al. (J. Clin. Pathol. **15**:544, 1962). Attempts to demonstrate this activity with broth culture filtrates of the chloramphenicol-resistant *S. typhosa* strains were unsuccessful.

The nature of the factor in the homogenate and its mechanism of inactivation of chloramphenicol are under study.