

The problem and implications of chloramphenicol resistance in the typhoid bacillus

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(Received 23 November 1974)

SUMMARY

Transferable chloramphenicol resistance has become common in the typhoid bacillus in countries such as Mexico, India, Vietnam and Thailand. Situations such as this, and others analogous to it in many parts of the world, are the result of the long-term indiscriminate use of chloramphenicol and other antibiotics in the affected areas. They can be rectified only by more rational antibiotic usage.

INTRODUCTION

The discovery of the efficacy of chloramphenicol in the treatment of typhoid fever was the most important therapeutic advance since the disease was first defined. The mortality of typhoid fever treated by the methods used before the advent of chloramphenicol was usually 10% or more. Such treatment consisted largely of nursing, symptomatic relief, and vigilance for the possibility of complications such as haemorrhage or intestinal perforation. Chloramphenicol transformed the scene, for it reduced the mortality of typhoid to 1% or less.

Unfortunately, the drug was used unnecessarily for the treatment of other diseases shortly after its introduction, and a number of cases of blood dyscrasia, some fatal, caused by chloramphenicol were reported (Erslev, 1964; Wallerstein *et al.* 1969; Report, 1971). Many practitioners, therefore, now hesitate to use it, even in typhoid fever. Substitutes for chloramphenicol have been proposed for the treatment of the disease, but time has shown that chloramphenicol remains the drug of choice. Moreover, blood dyscrasias have very rarely followed its use in typhoid, and the improved prognosis in that disease far outweighs the risk of damage by the drug to the haemopoietic system.

The main danger to be feared in the use of chloramphenicol for typhoid is thus not the risk of blood dyscrasia, but the appearance of chloramphenicol-resistant typhoid bacilli, an event that would impair or destroy its efficacy in the treatment of typhoid fever. In a recent report (Anderson & Smith, 1972) we described a chloramphenicol-resistant strain of the typhoid bacillus that caused an enormous outbreak of typhoid fever (> 10,000 cases) in Mexico in 1972-3. A number of foreign visitors to that country were infected: at least 52 Americans, two British and one Swiss (Anderson & Smith, 1972; Waldvogel & Pitton, 1973; Cohen, 1973).

The strain of *Salmonella typhi* that caused this epidemic had two important features: it was highly resistant to chloramphenicol, the minimal inhibitory concentration of the drug being of the order of 150 $\mu\text{g./ml.}$; and this resistance resulted from carriage of a transferable resistance factor (R factor) which coded not only for resistance to chloramphenicol (C), but also for resistance to streptomycin (S), sulphonamides (Su) and tetracyclines (T). This R factor belonged to a new plasmid compatibility group, which we originally designated H (Anderson & Smith, 1972; Grindley, Grindley & Anderson, 1972; Grindley, Humphreys & Anderson, 1973; Smith, Grindley, Humphreys & Anderson, 1973). Further work has resulted in the subdivision of this group into H₁ and H₂.

The prototype of group H₁ is the R factor identified in the strain of *S. typhi* that caused the Mexican typhoid outbreak (Grindley *et al.* 1972, 1973). Group H₂ is represented by a factor with the resistance markers CSSu, defined in a strain of *S. typhi* isolated from a patient infected in Spain. The H₂ subgroup has since been identified in many drug-resistant cultures of *S. typhimurium* isolated in Portugal, so that it appears to be common in the Iberian peninsula. We have also found it in *S. typhimurium* isolated in Belgium, Canada and Israel.

In this paper we shall concentrate principally on the distribution of group H₁ factors, mainly in *S. typhi*, but also in *S. typhimurium*.

PROPERTIES OF GROUP H RESISTANCE FACTORS

These factors belong to Class 1, that is, their resistance determinants and transfer factors are covalently bonded to form a single plasmid, which is transferred as an intact linkage group (Anderson & Threlfall, 1974).

H₁ and H₂ R factors are larger than most enterobacterial plasmids, with a molecular weight of about 120×10^6 daltons (Grindley *et al.* 1973).

Group H₁ factors transfer from *S. typhi* to *Escherichia coli* K12 (= K12), and thence into drug-sensitive *S. typhi*, with a frequency as low as 10^{-6} in overnight crosses at 37° C. Williams Smith (1974) has observed that this transfer frequency is greatly augmented at 22° C. and 28° C. We have confirmed this, and have shown that it applies also to the group H₂ R factor isolated from *S. typhi* originating in Spain, and described by Anderson & Smith (1972). Moreover, H₁ R factors from *S. typhimurium* in England and South East Asia, and H₂ factors from *S. typhimurium* in Portugal and Canada, all show a similar temperature-dependent transfer frequency. Transfer of these factors from *S. typhimurium* into K12 in overnight crosses at 28° C. occurs at a frequency approaching unity, and the same is true of their transfer from K12 into *S. typhi*. However, although the transfer frequency of H₁ and H₂ R factors from K12 into *S. typhimurium* is also much higher at 28° C. than at 37° C., it does not greatly exceed 10^{-2} .

We currently use accelerated transfer at 28° C. as opposed to 37° C. for the primary isolation of group H R factors from wild strains of enterobacteria.

Group H₁ R factors are not particularly stable in *S. typhi*, but may segregate to produce resistance-loss variants or completely sensitive lines of the organism with detectable frequency in a few days or weeks, in cultures stored at room temperature.

METHOD OF IDENTIFICATION OF H₁ R FACTORS

Technical details will not be given here, except for the description of a rapid test for the identification of group H₁ factors. This consists in the introduction of the plasmid into K12 in both the F⁺ and Hfr states. K12 F⁺ cells carry the F factor in the extrachromosomal state. In K12 Hfr cells, however, the F factor is integrated into the chromosome. H₁ factors are incompatible with the extrachromosomal F factor, which is displaced when they enter K12 F⁺ cells. The host strain thereby becomes immune to phage μ 2 (Dettori, Maccacaro & Piccinin, 1961), which specifically lyses cells carrying the F factor or factors related to it.

The integration of the F factor into the bacterial chromosome in K12 Hfr strains gives it security of tenure in the host cell, so that it cannot be displaced by the introduction of H₁ factors. As these factors are *fi*⁻, therefore, the K12 Hfr/H₁ hybrids exhibit visible lysis with phage μ 2. They are also stable, in contrast to K12 Hfr/F hybrids, which are highly unstable. These characters facilitate distinction between the F and other F-like factors, and those of group H₁. A further distinction is that phage μ 2 can be propagated on strains carrying the great majority of F-like factors, even when visible lysis with this phage is abolished by repression of the synthesis of F fimbriae, which are the receptors of phage μ 2 (Meynell & Datta, 1966*a*, *b*). Strains carrying H₁ plasmids alone will not support growth of phage μ 2, since they do not code for synthesis of F fimbriae. Complete identification of group H₁ factors depends on the demonstration of their incompatibility with the prototype H₁ factor in a standard host, and of molecular homology with this factor. These techniques, which are used in the routine identification of R factors and other plasmids in our laboratory (Anderson & Threlfall, 1974), will not be described here.

Group H₂ R factors are incompatible with H₁ factors, but their DNA shows no homology with that of H₁, although they are similar in transfer properties, in size, and in the percentage of guanine + cytosine in their DNA (Grindley *et al.* 1973; N. Grindley & E. S. Anderson, unpublished). H₂ factors are fully compatible with F, whether it is in the extrachromosomal or integrated state.

INTERNATIONAL DISTRIBUTION OF GROUP H₁ FACTORS
IN *S. TYPHI* AND *S. TYPHIMURIUM**In S. typhi*

The first strains of *S. typhi* carrying group H₁ resistance factors received by the Enteric Reference Laboratory belonged to the Mexican outbreak of 1972-3. Three of these cultures had been isolated in Europe. Vi-phage-typing revealed that they belonged to a degraded Vi-strain with a pattern of lysis characteristic of that associated with the large Mexican epidemic. All the strains we examined from this outbreak showed the same pattern of sensitivity to the Vi-typing phages, an indication that the epidemic was caused by a single line, that is, a clone, of the typhoid bacillus.

Paniker & Vimala (1972) described an outbreak of chloramphenicol-resistant typhoid in Kerala, India. We have studied 26 strains from this outbreak; they

Table 1. *Group H₁ R factors in Salmonella typhi*

Country of origin	Host	Phage type	Year of isolation	R-type*	No. examined in ERL
Mexico	Human	Degraded Vi-strain	1972	CSSuT	8
India	Human	D1-N	1972	CSSuT	26
S. Vietnam	Human	E7 (5)† D6 (8) N (1) 56 (32) Untypable Vi-strains (25)‡	1972-1974	CSSuT§	71
Thailand	Human	D1 (5) 53 (12) Vi-negative variant (1)	1973-1974	ACSSuT (5) CSSuT (12) CSSuT	18

* R-type = spectrum of drug resistance.

† Number of cultures shown in parentheses.

‡ Untypable with adapted Vi II phages, but showing specific reactions with other Vi phages. There were three distinct patterns with these 25 untypable strains: UVS1 (3); UVS2 (17); UVS3 (5).

§ One Vi-type 56 carries a CSSu group H₁ R factor.

Resistances: A = ampicillin; C = chloramphenicol; S = streptomycin; Su = sulphonamides; T = tetracyclines.

Table 2. Group H₁ R factors in *S. typhimurium* in South East Asia

Country of origin	Host	Phage type	Year of isolation	R-type	No. examined in ERL
Singapore	Human	193	1971	AKT (12) AT (5)	17
			1974	AKT (14) AT (4)	18
	Animal	193	1971-1972	AKT (20) AT (12)	32
Malaysia	Human	193	1972	AKT (11) AT (8)	19
			1973	AKT (16) AT (10)	27
	Animal	193	1973	AK (1) AT (1) AKT (1)	2

Resistances: A = ampicillin; K = neomycin-kanamycin; T = tetracyclines.

All but 5 cultures from Singapore and all but 2 cultures from Malaysia carried an independent SSu resistance determinant (see text).

belong to Vi-phage type D1-N, which again indicates that a clone of the typhoid bacillus was responsible for the outbreak. The strain carries a group H₁ R factor with the resistance spectrum CSSuT, similar to that of the R factor of the Mexican strain (Grindley *et al.* 1972, 1973).

Seventy-one cultures of chloramphenicol-resistant *S. typhi* were sent to us from South Vietnam in early 1974 (Butler, Linh, Arnold & Pollack, 1973). They belonged to the following seven types, clearly distinguishable with the Vi-typing phages: D6, E7, N, 56 (a new Vi-type), UVS1, UVS2 and UVS3. The designation UVS (= untypable Vi-strain) indicates a strain resistant to the adapted Vi-typing phages of Craigie & Yen (1938*a, b*), but showing specific patterns of lysis (1, 2 and 3) with a range of unadapted Vi-phages. All these cultures carry a group H₁ R factor showing the CSSuT resistance pattern (R-type), except for one type 56 culture, which carried an H₁ factor of R-type CSSu.

Eighteen chloramphenicol-resistant cultures of *S. typhi* from Thailand (Lampe, Mansuwan & Duangmani, 1974) reached us in 1974. Twelve belonged to Vi-type 53, five to type D1, and one was a Vi-negative variant resistant to all Vi-phages. The type 53 cultures and the Vi-negative variant carry an H₁ R factor with the R-type CSSuT, and the type D1 carries a factor of the same group, but with the R-type ACSSuT (A = ampicillin). These findings are shown in Table 1.

In *S. typhimurium*

We have recently identified group H₁ R factors in phage type 193 of *S. typhimurium* isolated from outbreaks of animal and human infection in Singapore and Malaysia. They code for AT, AK (K = kanamycin) and AKT resistances, and the Singapore and Malaysian R factors are indistinguishable from each other.

A single strain of phage type 3 of *S. typhimurium* of human origin carrying a group H₁ R factor coding for tetracycline resistance only was isolated in Britain and defined in the Enteric Reference Laboratory in 1961. The R factor carried by this strain was identified as belonging to group H after the group had been described (Grindley *et al.* 1972), and it has since been assigned to the H₁ subgroup.

Table 3. Group H_1 R factors in *S. typhimurium* in the United Kingdom

Host	Phage type	Year of isolation	R-type	No. examined in ERL
Human	3	1961	T (1)*	1
Human	193	1971	AKT (1) AT (2)	3
Human	193	1972	AKT (9) AT (5)	14
Human	193	1973	AKT (2)* AT (2)* AT (1)	5
Human	193	1974	AT (22)	22

* All cultures except these carried an independent SSu resistance determinant (see text).

A retrospective survey of 22 strains of phage type 193 of *S. typhimurium* isolated in Britain in 1971 (3), 1972 (14) and 1973 (5), revealed that they carried group H_1 R factors with the R-type AT or AKT. Four of the patients, two in 1971 and two in 1972, had been infected in South East Asia. The remaining infections, of which the source was unknown, had been contracted in the United Kingdom. Twenty-one cases of salmonellosis in an outbreak of unknown origin in Northern Ireland in 1974 were caused by the same phage type of *S. typhimurium*, which carried an H_1 R factor with the R-type AT. An isolated case in England was caused by a similar strain of *S. typhimurium* in the same year.

All but seven of the *S. typhimurium* cultures from South East Asia, and all but five of those from the United Kingdom, also carried an independent determinant for streptomycin and sulphonamide resistance, the transfer of which was mediated by the H_1 factor.

Our findings with these cultures are summarized in Tables 2 and 3.

A chloramphenicol-resistant culture of *S. typhi* was recently received from Algeria. The strain belongs to Vi-type 44 and carries an R factor coding for ACKSSuT resistances. This R factor is fi^+ and belongs to compatibility group F_1 , that is, it is incompatible with the F factor of *E. coli* K12 and codes for the synthesis of F fimbriae. It converts type A of *S. typhi* into type 44. Investigation of the type strain of *S. typhi* type 44, which was defined in 1958 and is drug-sensitive, revealed that it carries a transfer factor similar to that in the resistant strain. Carriage of this transfer factor is in fact responsible for the specific Vi-phage typing pattern that characterizes Vi-type 44 of *S. typhi*. Possession of such a transfer factor could have enabled the respective strain of the typhoid bacillus to acquire its transferable drug resistance by the mechanism previously described (Anderson, 1965), that is, passage of the transfer factor into a strain carrying an ACKSSuT resistance determinant; and recombination of the transfer factor with the determinant to form the new R factor, which is subsequently transferred to the typhoid bacillus. This sequence of events would be promoted by the use of any of the antibiotics in the ACKSSuT resistance spectrum.

A similar though not identical fi^+ F_1 ACSSuT resistance factor in the typhoid bacillus has been described in France by Chabbert & Gerbaud (1974).

DISCUSSION

Distribution of H₁ resistance factors

Although the largest outbreak of chloramphenicol-resistant typhoid fever (> 10,000 cases) occurred in Mexico, an impressive feature of our observations is the prevalence of chloramphenicol resistance due to group H₁ R factors in *S. typhi* isolated in South East Asia; a prevalence which is apparently increasing. Naturally, only a very small sample of cultures has been examined. With the exception of that isolated from the Vi-type D1 culture from Thailand, and a CSSu factor in one culture of type 56, all the H₁ R factors involved code for CSSuT resistance. The ACSSuT factor found in the type D1 culture could have been formed by the addition of a locus coding for ampicillin resistance to a CSSuT factor, so that there may basically be only a single widely distributed group H₁ R factor in *S. typhi* in these regions. On the other hand, CSSuT resistance linkage groups are common, and may simply reflect antibacterial drugs frequently used in man in the areas concerned. The alternative hypothesis can thus be advanced that group H₁ transfer factors are widespread in southern Asia, and that they have acquired determinants for resistance to the most commonly used antibiotics. The addition of ampicillin resistance to the factor isolated from the type D1 strain of *S. typhi* from Thailand does not conflict with this hypothesis, since ampicillin is also widely used, particularly in chloramphenicol-resistant typhoid fever. It would therefore be expected that, in an environment where plasmid-borne ampicillin resistance is common, strains of chloramphenicol-resistant *S. typhi* might eventually become resistant also to ampicillin, either by the insertion of an ampicillin resistance locus into existing CSSuT R factors, or by the acquisition of a new R factor coding for ampicillin resistance. An example of the first mechanism is described above. Acquisition of an R factor for ampicillin resistance was reported by Cohen (1973) in a case of infection with the Mexican chloramphenicol-resistant strain treated with ampicillin. The strain acquired an additional *fi*⁺ group I₁ factor coding for ampicillin and kanamycin resistances. This factor was distinct from and compatible with the H₁ R factor already carried by the typhoid strain.

The Mexican typhoid outbreak was caused by a single strain of the typhoid bacillus and, so far as we know, only one strain of Vi-type D1-N was concerned in the Indian outbreak. But South Vietnam and Thailand have so far yielded nine Vi-types of *S. typhi* carrying group H₁ R factors. The types concerned are quite distinct and are therefore almost certainly independent of each other. Even in these countries of high typhoid incidence it is inconceivable that different Vi-types of *S. typhi* are common enough to reach the concentrations required for direct transfer of R factors to occur between them. The virtual certainty must therefore be accepted that each strain of the Vi-types represented has acquired its H₁ R factor in a discrete event, probably from non-pathogenic intestinal organisms such as *E. coli*, in which these factors must be prevalent in the areas concerned. The numbers of the chloramphenicol-resistant Vi-types of *S. typhi* received suggest that each type is distributed on a substantial scale.

The presence of H₁ R factors coding for AK, AT and AKT resistances in the same phage type of human and animal *S. typhimurium* in Singapore and Malaysia indicates that the resistant strains have a common origin. Ordinarily, the direction of spread would be accepted as being from animals to man, but environmental conditions in South East Asia may also facilitate transmission in the opposite direction. Most of the type 193 cultures with these H₁ factors are also resistant to streptomycin and sulphonamides (SSu). The SSu determinant is carried in a Class 2 relationship by the respective H₁ factors, that is, is transferred as a separate plasmid which retains its independence in the new host (Anderson & Threlfall, 1974). Although four of the British infections with *S. typhimurium* carrying AT and AKT group H₁ R factors were contracted in South East Asia, the remainder were acquired in the United Kingdom and their origin is so far undetermined.

The cause of the rise of chloramphenicol resistance in the typhoid bacillus is unfortunately only too evident. It is the result of the widespread, protracted and indiscriminate use of chloramphenicol and other antibiotics in man and animals. In developing countries such as Mexico, India, Vietnam and Thailand it may have arisen primarily in man. In such countries there are no restrictions on the sale of antibiotics. Not only are they used imprudently by doctors, therefore, but they are also freely available from pharmacists to the general public. This has resulted in a high incidence of non-pathogenic enterobacteria carrying R factors. Only one resistance transfer event from such an organism to a single typhoid bacillus suffices to produce a strain which, if it is provided with the opportunity for transmission, will precipitate an epidemic of chloramphenicol-resistant typhoid fever with its attendant difficulties in treatment (Anderson & Smith, 1972). The present risk of such an event is small in developed countries with their low typhoid incidence. But the proof of the postulate is evident in Mexico, and in South East Asia it is clear that transfer to the typhoid bacillus of H₁ R factors coding for chloramphenicol resistance has happened many times. And because the R factors concerned code for multiple drug resistance, the strains carrying them can be favoured by any of the drugs represented in their resistance spectrum: chloramphenicol, streptomycin, sulphonamides and tetracyclines in those carrying the CSSuT factors; and ampicillin in addition in the ACSSuT factor from Thailand. Moreover, such resistant strains may spread to countries of normally low typhoid incidence, as happened with the Mexican typhoid strain, and they may establish themselves in those countries.

The reason for the prevalence of H₁ R factors in the typhoid bacillus is not yet apparent. The accelerated transfer of these factors below 30° C. (Williams Smith, 1974) may facilitate transmission of the factors from non-pathogenic enterobacteria to the typhoid bacillus outside the body. However, the solution of this problem awaits further study.

General observations on the distribution of R factors

The subject of R factor-mediated drug resistance in enterobacteria in general and in pathogenic enterobacteria in particular, can now be viewed in perspective, because much work has been done in this field since R factors were first discovered (Ochiai, Yamanaka, Kimura & Sawada, 1959; Akiba *et al.* 1960). It has been established that these plasmids are now abundant in the enterobacteria of man and livestock throughout the world. This massive shift in the ecology of the enterobacteria of man is the result of the use of antibacterial drugs for the treatment of disease in man caused by enterobacterial and other, often unidentified, pathogens. In animals it is the consequence of the use of antibiotics as feed additives and for the prophylaxis and treatment of disease. Moreover, it is now clear that the R factors in man and animals are drawn from a common pool (Anderson, Threlfall, Carr & Savoy, 1973; Anderson & Threlfall, 1974; Smith, Humphreys & Anderson, 1974; Anderson, Humphreys & Willshaw, in preparation).

The enterobacteria in which transferable drug resistance first emerges are ordinary intestinal commensals such as *E. coli*. They are usually non-pathogenic but are highly communicable. They therefore spread to the intestine of other human or animal hosts, irrespective of the use of antibiotics, but their prevalence is sustained by the incessant use of these drugs. From time to time their resistance is transferred to a pathogen which subsequently causes an outbreak of infection. The result is an epidemic of a disease which is no longer susceptible to drugs that might formerly have been used for its treatment. The widespread appearance of chloramphenicol-resistant typhoid fever, of intractable and virulent outbreaks of infection with multiresistant salmonellas in paediatric units in South America and elsewhere (Anderson, 1974), of epidemic drug-resistant *Shigella dysenteriae* 1 infection in Central America (Mata, Mendizabal-Morris, Gangarosa & Perera, 1970), and of problems of hospital infection with resistant opportunist enterobacterial pathogens in many countries, are worrying examples of this. But such events were predictable, and are yet another warning that rationalization and reduction of the use of antibiotics and other antibacterial drugs are necessary, not only in the developing countries but in the world at large. The medical and veterinary professions bear a heavy responsibility in this respect, as do pharmaceutical manufacturers, who should be watchful for the misuse of their products, and should conduct promotional campaigns with a real awareness of the dangers arising from their abuse. The time has clearly come when international co-operation at legislative and professional levels is needed to attempt to reverse the change in the ecology of the enterobacteria and other organisms that has resulted from the indiscriminate use of antibacterial drugs.

I thank the following for sending chloramphenicol-resistant cultures of *S. typhi*: Dr C. K. J. Paniker of Kerala, India; Dr T. Butler of Baltimore, U.S.A. (cultures from Vietnam); Professor Vu Qui Dai of Saigon, Vietnam; Major R. M. Lampe of San Francisco, U.S.A. (cultures from Thailand) and Professor B. Mered of Algeria. Drug-resistant *S. typhimurium* cultures were kindly provided by

Dr Moses Yu and Mr Liow T.M. of Singapore, and Dr Bhagwan Singh of Kuala Lumpur, Malaysia.

My thanks are also due to Mr H. R. Smith, Dr E. J. Threlfall, Mrs J. Frost and Dr L. G. Savoy for assistance in this work, which was partly supported by a grant from the Department of Health.

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