CHLORAMPHENICOL RESISTANCE IN *ESCHERICHIA COLI*

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ONE consequence of the discovery that much of the antibiotic resistance of enterobacteria is transmissible has been concern about the chloramphenicol-resistance status of the non-pathogenic *Escherichia coli* that inhabit the alimentary tract of man and domestic animals. This is principally because of the possibility that such resistance might ultimately be transmitted to *Salmonella typhi* and so limit the usefulness of chloramphenicol in the treatment of typhoid fever.

To demonstrate the transfer of R factors *in vivo* requires the use of donor and recipient organisms that not only colonise the alimentary tract well but that transfer the factors from one to the other at a high rate *in vitro* (Smith, 1970). In most in-vitro studies, *E. coli* strain K12F⁻ is employed to determine whether the antibiotic resistance possessed by strains of enterobacteria is transmissible; whether the results so obtained can be taken as an indication of the extent and rate at which the R factors involved would be transmitted to other strains is questionable. In view of this and of the importance of transmissible chloramphenicol resistance, it was decided to compare strain K12F⁻ with a number of pathogenic and non-pathogenic enterobacteria in regard to their ability to receive R factors from a collection of strains of *E. coli* that were chloramphenicol-resistant.

MATERIALS AND METHODS

Chloramphenicol-resistant strains of Escherichia coli. Of these, 400 had been isolated from the faeces of domestic animals, principally calves, kept on different premises and 280 had been isolated from different specimens of human sewage.

Potential recipient strains. These were nalidixic acid-resistant mutants (*nal*^r), prepared in the laboratory by the method of Smith and Gyles (1970) from ten fully antibiotic-sensitive strains. These were *Salmonella typhimurium* of phage-type 29, *Salm. typhi* Vi⁺, *Salm. heidelberg*, *Shigella flexneri*, *Sh. sonnei*, two enteropathogenic strains of *E. coli* of serotype O26:K60:H— and O55:K59:H6 and two untyped non-pathogenic strains of this organism that had been isolated from the faeces of healthy human beings, nos. 124 and 126. Of these ten strains, only one, *E. coli* no. 124, was found to possess a transfer factor.

Antibiotic sensitivity tests. These were performed by the disk-diffusion method used by Smith (1970). The minimum inhibitory concentration of chloramphenicol for different strains of *E. coli* was determined by the method of Smith (1966).

The identification of strains possessing transfer factors only or resistance determinants only. This was achieved by the mobilisation test of Anderson (1965), as described by Smith and Linggood (1970).

Determination of degree of transmission of chloramphenicol resistance. Nutrient Broth

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(Oxoid no. 2) in 10-ml amounts was seeded with 0.02 ml of a broth culture of a prospective donor strain and an equal amount of a similar culture of a *nal*^r prospective recipient strain. The mixed culture was incubated in a waterbath at 37°C for 24 hr and the numbers of organisms that had acquired the chloramphenicol R factor from the donor strain assessed by performing viable counts on MacConkey agar containing 20 µg per ml each of sodium nalidixate and of chloramphenicol. In the experiments in which *E. coli* strains K12F⁻, H124 and H126 and *Salm. typhimurium* were recipients, the counting method employed was that of Miles and Misra (Miles, Misra and Irwin, 1938), the counts being duplicated on MacConkey medium containing sodium nalidixate only so that the total number of prospective recipient organisms present could also be assessed. In the experiments in which the other prospective recipients were used, the number of organisms that had acquired the

TABLE I

Antibiotic-resistance patterns of 670 strains of Escherichia coli selected because they were resistant to chloramphenicol

Resistance pattern	Percentage of strains	Resistance pattern	Percentage of strains
A S Su T C	37.4	S Su N C	0.6
S Su T C	16.2	S Su C F	0.6
A S Su N T C	11.9	A C	0.6
S Su N T C	9.5	A S T C	0.5
S Su C	7.9	S Su N T C F	0.3
A S Su C	4.9	A S Su C F	0.3
A S Su N T C F	2.5	A S Su N T C Na	0.2
A T C	2.2	A S Su C Na	0.2
A S Su T C F	1.4	S T C	0.2
S Su T C F	1.4	A Su C	0.2
A S Su N C	1.0	C	0

A = Ampicillin; S = streptomycin; Su = sulphonamides; N = neomycin; T = tetracyclines; C = chloramphenicol; F = furazolidone; Na = nalidixic acid.

chloramphenicol R factor was assessed by streaking 0.02 ml of the mixed culture, undiluted and diluted 200 times, in a standard manner on the MacConkey medium containing sodium nalidixate and chloramphenicol and comparing the amount of growth that occurred after incubation with that obtained from mixed cultures containing different known concentrations of chloramphenicol-resistant organisms. The total number of prospective recipient organisms in the mixed cultures varied little. When the rate of transfer of chloramphenicol resistance was high, a full count was performed by the Miles and Misra method.

Fertility inhibiting (fi) character of R factors. This was assessed by transferring the chloramphenicol R factor into a K12F⁺ strain and then examining for susceptibility to lysis by the F-specific phage MS2 (Davis, Strauss and Sinsheimer, 1961).

RESULTS

The results of determining the antibiotic-resistance patterns of 670 chloramphenicol-resistant strains of *E. coli* of human or animal origin are shown in table I. None of the strains was resistant to chloramphenicol only; over 66 per cent. of them were resistant to five or more of the eight antibiotics against which they were tested. In all, 97 per cent. were resistant to streptomycin, 96.5 per cent. to sulphonamides, 83.7 per cent. to tetracyclines, 63.3 per cent. to ampicillin, 26.0 per cent. to neomycin, 6.5 per cent. to furazolidone and 0.4 per cent. to sodium nalidixate. Eighteen strains, selected because most

of them possessed different antibiotic-resistance patterns, were also tested against spectinomycin; 14 of them were resistant. The minimum inhibitory concentration of chloramphenicol for 100 strains representing 17 different antibiotic-resistance patterns was 400–800 μg per ml; the corresponding figure for strains classed by disk tests as chloramphenicol sensitive was 2 μg per ml.

There was considerable variation in the extent and the rate of transfer of chloramphenicol resistance from *E. coli* strains of animal origin to three strains of *Salmonella*, two of *Shigella* and five of *E. coli*, the latter including two human non-pathogens (nos. 124 and 126) and two enteropathogens (table II). *E. coli* strain K12F⁻ was the best recipient and the enteropathogenic *E. coli* O55:K59:H6 strain the worst. All the *E. coli* strain K12 organisms

TABLE II

The transfer rate of chloramphenicol resistance from different potential donor strains of Escherichia coli to different potential recipient strains of enterobacteria

Potential recipient strain	Number of potential donor strains with which i was tested	Percentage of potential donor strains whose chloramphenicol resistance was acquired by the following percentage of organisms of the potential recipient strain*							
		>10 ¹	>10 ⁰	>10 ⁻¹	>10 ⁻²	>10 ⁻³	>10 ⁻⁴	>10 ⁻⁵	>10 ⁻⁶
<i>E. coli</i> no. K12F ⁻	400	52	67	73	77	80	87	88	96
<i>Salm. heidelberg</i>	160	10	28	48	62	68	73	...	...
<i>Salm. typhi</i> Vi ⁺	40	0	8	35	68	80	93	...	...
<i>Sh. flexneri</i>	40	0	15	33	45	53	78	...	...
<i>E. coli</i> no. 124	200	3	13	40	42	55	66	...	...
<i>Sh. sonnei</i>	150	4	10	37	48	61	67	...	...
<i>Salm. typhimurium</i>	400	0.3	4	11	18	36	50	55	56
<i>E. coli</i> no. 126	185	0.5	1	5	15	33	46	...	...
<i>E. coli</i> O26:K60:H—	375	0.3	1	3	9	16	23	46	52
<i>E. coli</i> O55:K59:H6	400	0	0	1	1	1	3	7	13

* = During mixed culture for 24 hr at 37°C.

... = Insufficient culture examined to detect transfer to less than 10⁻⁴ per cent. of organisms.

appeared to have received the chloramphenicol R factor in the tests with 20 per cent. of the prospective donor strains. The corresponding figure for *Salm. heidelberg* was 1.5 per cent.; this high transfer rate was not noted in the case of any of the other eight potential recipient strains.

The antibiotic resistance possessed by two of the 16 strains that did not transfer their R factors to strain K12F⁻ could be mobilised by transfer factors F and I. In seven of ten donor strains studied in more detail, all the R determinants appeared to be linked. Eight of the ten strains possessed fi⁺ transfer factors, one an fi⁻ transfer factor and one both kinds of transfer factors.

DISCUSSION

Because all the 670 apparently unrelated chloramphenicol-resistant strains of *Escherichia coli* were multi-resistant, the presence of chloramphenicol in

the alimentary tract might not be essential to favour either their survival in that organ or that of any other strains to which they transfer their R factors. Any of the other antibiotics to which they were resistant would probably suffice. In fact, those that, unlike chloramphenicol, are excreted in the faeces in high concentration after oral administration would be expected to function better in this respect. This was actually the case in studies on the experimental transfer of R factors *in vivo* (Smith, 1970).

The results confirm the well-established usefulness of *E. coli* strain K12F⁻ in detecting strains of *E. coli* whose antibiotic resistance is due to R factors in that all except 16 of 400 of the chloramphenicol-resistant strains transmitted their resistance to it, often at a very high rate. The results with this strain, however, gave little guide as to the rate at which the R factors would be transmitted to the other strains that were either pathogens or might be concerned in the epidemiology of R factors. It is important to know this because, in experimental studies, R-factor transfer could usually be detected *in vivo* only between those combinations of strains that transmitted and received R factors at a high rate *in vitro* (Smith, 1970).

SUMMARY

All of 670 apparently unrelated chloramphenicol-resistant strains of *Escherichia coli* of human and animal origin were multiply-resistant; over 66 per cent. of them were resistant to five or more antibiotics.

The resistance of 96 per cent. of 400 of the strains could be transmitted, often at a high rate, to *E. coli* strain K12F⁻. A lower and variable proportion of the strains transmitted their resistance to nine other enterobacterial strains, principally pathogens; the rate of transmission was much lower than that which occurred to *E. coli* strain K12F⁻.

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