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The occurrence of plasmids carrying genes for both enterotoxin production and drug resistance in *Escherichia coli* of human origin

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SUMMARY

Twenty-three of 89 enterotoxigenic strains of *Escherichia coli* were resistant to one or more antimicrobial agents. Eleven strains transferred resistance directly and five transferred resistance after mobilization. In three cases a resistant recipient was enterotoxigenic. One of these strains contained a conjugative plasmid carrying genes for both drug resistance and enterotoxin production. In the two other strains genes for drug resistance and enterotoxin production were carried on separate co-transferable plasmids.

INTRODUCTION

Drug-resistant, enterotoxigenic strains of *Escherichia coli* have been reported by several authors (Merson *et al.* 1976; Sack *et al.* 1978; Echeverria *et al.* 1978). Since the genes controlling enterotoxin production, like those responsible for drug resistance, may be carried by plasmids (Gyles, So & Falkow, 1974), it is possible that plasmids might arise in nature which carry genes for both these characters. One such plasmid has been reported in a strain of *E. coli* of porcine origin (Gyles, Palchaudhuri & Maas, 1977).

The occurrence of plasmids carrying genes for both drug resistance and enterotoxin production in strains of *E. coli* causing human diarrhoeal disease might have important implications in the treatment and prevention of the disease. We searched for such plasmids in enterotoxigenic *E. coli* strains of human origin from numerous geographical areas.

MATERIALS AND METHODS

Bacterial strains

Eighty-nine strains of *E. coli* from 14 countries were included in the study (Table 1). They had been isolated from faecal specimens of patients with diarrhoea. All the strains were enterotoxigenic. Fifty strains produced both heat stable enterotoxin (ST) and heat labile enterotoxin (LT), 24 strains produced ST only and 15 produced LT only (Table 1).

Two F^- *E. coli* K12 strains were used as recipients in transfer experiments:

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Table 1. *Enterotoxigenic* E. coli *strains tested for drug resistance*

O serogroup	No. of *E. coli* strains tested	No. of drug-resistant strains*	Enterotoxins produced		
			ST	LT	ST and LT
O1	1	0	—	—	1
O6	20	3	—	2	18
O7	1	1	—	1	—
O8	9	0	—	1	8
O15	1	0	—	1	—
O20	2	0	1	—	1
O25	2	0	—	—	2
O27	3	2	3	—	—
O60	1	0	—	—	1
O63	3	1	2	—	1
O78	7	3	1	1	5
O85	1	0	—	—	1
O89	1	0	1	—	—
O114	3	1	1	—	2
O115	5	0	—	—	5
O128	3	3	2	1	—
O148	7	2	5	—	2
O153	2	1	2	—	—
O159	4	1	1	2	1
O rough	5	3	2	2	1
O?	8	2	3	4	1
Total	89	23	24	15	50

* The following drugs were used: ampicillin (25), cephaloridine (30), chloramphenicol (10), gentamicin (10), nalidixic acid (30), neomycin (10), nitrofurantoin (30), streptomycin (10), sulphathiazole (100), tetracycline (10), trimethoprim (1·25). Concentrations in μg/ml.

J53-1 *pro met* nalidixic acid-resistant (Nal^r) and IR716 *lac his pro trp* streptomycin-resistant (Sm^r).

Four *E. coli* K12 strains carrying transfer factors were used as donors in mobilization experiments. The F-like factors used were F-T (Anderson *et al.* 1977) and R1-16 (Meynell & Cooke, 1969) which code for resistance to tetracycline and kanamycin respectively. The I-like factors were T-Δ *drp* (Grindley & Anderson, 1971) and R144-3 (Meynell & Cooke, 1969) which code for tetracycline and kanamycin resistance respectively.

Serotyping

The strains of *E. coli* were serotyped using antisera for O groups O1 to O164 and for flagellar antigens H1 to H56 (Ørskov & Ørskov, 1975).

Tests for colicinogeny and drug resistance

The 89 strains were tested for colicinogeny by the method of Fredericq (1957) and were tested for sensitivity to antibacterial agents using an agar plate dilution technique (Haltalin, Markley & Woodman, 1973).

Tests for transfer of drug resistance

Transfer of drug resistance to the nalidixic acid-resistant strain J53-1 was tested by inoculating 0·1 ml of overnight cultures of donor and recipient strains into 10 ml Hedley-Wright broth and incubating the mixture for 24 h at 37 °C. Mating mixtures were then plated on DST agar containing nalidixic acid, 50 μg/ml and appropriate drugs to select transconjugants: ampicillin, 100 μg/ml; cephaloridine, 10 μg/ml; chloramphenicol, 10 μg/ml; kanamycin, 30 μg/ml; streptomycin, 10 μg/ml; sulphathiazole, 100 μg/ml; tetracycline, 10 μg/ml. In one test the streptomycin-resistant strain IR716 was used as a recipient and mating mixtures were plated on DST agar containing streptomycin, 500 μg/ml and any appropriate drugs as above.

For mobilization tests, 0·1 ml of overnight cultures of a donor strain carrying a transfer factor and a drug-resistant *E. coli* strain were inoculated into 10 ml broth. After incubation for 24 h, 0·1 ml of this mixed culture and 0·1 ml of an overnight culture of the final recipient, J53-1, were inoculated into 10 ml broth and incubated 24 h. The final culture was plated on selective media as described above. In one mobilization test a strain carrying the non-conjugative plasmid NTP11, coding for kanamycin resistance, was used.

Tests for enterotoxin production

Production of ST was determined by the infant mouse test of Dean *et al.* (1972) and production of LT by the Y1 cell (Donta, Moon & Whipp, 1974) and CHO cell (Guerrant *et al.* 1974) tissue culture tests.

Tests for plasmid content

The plasmid content of certain strains was determined by agarose gel electrophoresis (Meyers *et al.* 1976) or by electron microscopy (Grindley, Humphreys & Anderson, 1973).

RESULTS

Serotypes and drug resistance

Seventy-six of 89 enterotoxigenic strains of *E. coli* belonged to 40 different O:H serotypes in 19 recognized O serogroups (Table 1). The remaining 13 strains were either auto-agglutinable (O rough) or did not belong to O groups O1 to O164(O?).

Twenty-three of the 89 strains were resistant to one or more antibacterial agents (Table 1).

Transfer and mobilization tests

Eleven of the 23 resistant strains transferred drug resistance to J53-1 directly. In each case a proportion of the transconjugants was resistant to all the antibacterial agents to which the donor strain was resistant (Table 2). One such transconjugant from each of the 11 mating mixtures was tested for enterotoxin production. Only one was enterotoxigenic (ST and LT), and this transconjugant

Table 2. *Toxigenic* E. coli *strains with transferable drug resistance factors*

Serotype	Origin	Enterotoxins produced: ST	Enterotoxins produced: LT	Colicinogeny	Drug resistance
O27.H7	Cruise ship	+	−	−	Tc
O27.H7	Canada	+	−	−	Tc
O63.H12	Bangladesh	+	−	−	Tc
O78.H12	South Africa	+	−	−	Ap Sm Su Tc
O128.H18	U.K. ex India	+	−	−	Tc
O128.H27	South Africa	+	−	−	Ap Sm Su Tc
O148.H28	Vietnam	+	+	−	Cm Sm Su Tc
O148.H28	Dubai	+	−	−	Sm Su
O153.H10	Bangladesh	+	−	−	Cm Sm Su Tc
O159.H34	Canada	+	+	+	Ap Cr
O rough H27	South Africa	+	−	−	Ap Sm Su Tc

Total 11 strains

Symbols for drug resistances: Ap, ampicillin; Cm, chloramphenicol; Cr, cephaloridine; Sm, streptomycin; Su, sulphathiazole; Tc, tetracycline.

Table 3. *Toxigenic* E. coli *strains with mobilizable drug resistance factors*

Serotype	Origin	Enterotoxins produced: ST	Enterotoxins produced: LT	Colicinogeny	Drug resistance	Mobilizing plasmid
O6.H16	Hong Kong	+	+	−	Sm Su	T-Δ *drp*
O6.H16	Aden	+	+	−	Su	F-T and T-Δ *drp*
O7.H18	U.K. ex India	−	+	−	Sm Su Tc	R144-3
O78.H12	India	+	+	−	Sm Su	F-T and T-Δ *drp*
O?H27	South Africa	+	−	−	Sm Su	F-T

Total 5 strains

Table 4. *Toxigenic* E. coli *strains from which resistance was not transferred*

Serotype	Origin	Enterotoxins produced: ST	Enterotoxins produced: LT	Colicinogeny	Drug resistance
O6.H16	Aden	+	+	−	Su
O78.H12	South Africa	+	+	−	Sm Su
O114.H21	South Africa	+	−	−	Sm Su
O128.H49	South Africa	−	+	−	Sm Su
O rough H10	South Africa	−	+	−	Sm Su
O rough H21	South Africa	+	+	+	Sm Su
O?H⁻	South Africa	+	+	+	Sm Su

Total 7 strains

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Table 5. E. coli *strains from which drug resistance and toxin production were transferred together*

Strain no.	Serotype	Enterotoxins produced	Drug resistance	Molecular sizes of plasmids present ($\times 10^6$ daltons)
E2985/76	O159.H34	ST and LT	Ap Cr	53 43 5·6 3·6 2·7
E7476/77	O?H27	ST	Sm Su	97 4·3
E5798/76	O7.H18	LT	Sm Su Tc	80 61 4·0 3·5

had acquired resistance to ampicillin (Ap^r) and cephaloridine (Cr^r) from strain number E2985/76 (*E. coli* O159.H34).

A further five strains transferred resistance after mobilization (Table 3) and resistant recipients were tested for enterotoxin production. Two strains gave resistant recipients which were enterotoxigenic. Strain number E7476/77 (*E. coli* O?H27) transferred resistance to sulphathiazole (Su^r) and streptomycin (Sm^r) together with ST production and strain number E5798/76 (*E. coli* O7.H18) transferred either Su^r and Sm^r or tetracycline resistance (Tc^r), together with LT production.

Seven strains failed to transfer resistance either directly or after mobilization (Table 4).

Plasmid studies

The transfer of plasmids from the three enterotoxigenic *E. coli* strains which had transferred resistance together with the ability to produce enterotoxins was studied in more detail. In addition the plasmid content of these strains and that of selected recipients was examined.

Strain number E2985/76, *E. coli* O159.H34, produced ST and LT, was Ap^r and Cr^r and was colicinogenic. It contained plasmids of five different sizes (Table 5). After incubation with *E. coli* J53-1 for 24 h, selection on ampicillin (500 μg/ml) showed that Ap^r had been acquired by recipient bacteria at a frequency of 3×10^{-3} per recipient cell. All the recipients which were Ap^r were also Cr^r. Seventy of 71 resistant recipients which were examined produced LT. Twenty-eight of these LT-producing recipients were also tested for ST, with positive results in all cases; only 22 of these 28 were colicinogenic. Enterotoxigenic, non-colicinogenic Ap^r recipients carried a single plasmid with a molecular weight of $54{\cdot}6 \times 10^6$ daltons measured by electron microscopy. This single plasmid coded for ST, LT and Ap^r. A transconjugant carrying this plasmid was able to transfer all three properties as a single linkage group to strain IR716 at a frequency of $1{\cdot}8 \times 10^{-2}$ per recipient cell in 1 h. Enterotoxin testing of recipients which were not selected for Ap^r showed that the ability to produce ST and LT had been acquired without Ap^r at a frequency of 3×10^{-2} per recipient cell. Examination of the plasmid content of such recipients showed that a single plasmid coded for ST and LT production without Ap^r; it had a molecular weight of $50{\cdot}4 \times 10^6$ daltons measured by electron microscopy. Further studies of the parent strain and the resistant,

enterotoxigenic recipients, which will be reported elsewhere (McConnell *et al.* 1979), suggested that transposition had occurred from a small ($5{\cdot}6 \times 10^6$ daltons) plasmid coding for Ap^r to the enterotoxin plasmid.

Strain number E7476, *E. coli* O?H27 produced ST only, was Sm^r and Su^r and contained plasmids of two sizes (Table 5). Sm^r and Su^r were mobilized together at a low frequency (10^{-8}) by plasmid F-T and the three recipients which had acquired drug resistance were also ST-producing. A spontaneously occurring drug sensitive enterotoxigenic variant of the parent strain was found to contain a single plasmid of molecular weight 97×10^6 daltons suggesting that the $4{\cdot}3 \times 10^6$ dalton species coded for Sm^r and Su^r. Further tests using strain E7476/77 as a donor strain showed that the ST plasmid was conjugative and could mobilize NTP11, a non-conjugative plasmid coding for kanamycin resistance, without transfer of Sm^r and Su^r. Transconjugants carried the 97×10^6 dalton plasmid confirming that it coded for ST production and not drug resistance.

Strain E5798/76, *E. coli* O7H18, produced LT only and was Sm^r, Su^r and Tc^r. It contained plasmids of four sizes (Table 5). All the resistances were mobilized at a low frequency (10^{-8}) by plasmid R144-3, recipients acquiring either Sm^r and Su^r, or Tc^r. In both cases only a proportion of resistant recipients were enterotoxigenic. Examination of enterotoxigenic, resistant recipients showed the presence of a 60×10^6 dalton plasmid which was absent from non-enterotoxigenic, resistant recipients. There was no evidence for linkage of the genes for toxin production and drug resistance in these transconjugants. When the direct transfer of enterotoxin production from strain E5798/76 to J53-1 without the introduction of a mobilizing plasmid was tested, LT production was transferred, without drug resistance, at the high frequency of 5×10^{-1}. These enterotoxigenic recipients had acquired only a 60×10^6 dalton plasmid confirming that it coded for LT production and not drug resistance.

DISCUSSION

Twenty-three of 89 enterotoxigenic *E. coli* strains were drug-resistant. Although the number of strains tested from each country was too small for detailed geographical comparison, only two of 21 strains tested from Bangladesh were resistant, whereas 10 of 17 strains from South Africa were resistant. Eleven strains transferred drug resistance directly to *E. coli* J53-1 and a further five transferred resistance after mobilization. One of the eleven strains which transferred drug resistance directly contained a plasmid which carried genes for both Ap^r and enterotoxin production (ST and LT). It seems likely that this strain contained some cells with plasmids carrying genes for enterotoxin production and Ap^r separately and others with a plasmid carrying genes for both characters together. The evidence suggests that Ap^r had transposed from a small ($5{\cdot}7 \times 10^6$ daltons) plasmid to a larger ($50{\cdot}4 \times 10^6$ daltons) enterotoxin plasmid to form a single large ($54{\cdot}6 \times 10^6$ daltons) plasmid and that recipients carrying this plasmid were selected by our experimental procedure.

Two other strains transferred drug resistance and enterotoxin production together following mobilization but the determinants proved to be carried by

separate plasmids. In both strains the enterotoxin plasmid was conjugative but we were unable to detect mobilization of the drug resistance plasmid by the enterotoxin plasmid. In this study only one resistant recipient from each mating was tested for enterotoxin production so that strains which transferred drug resistance and enterotoxin production together to only a proportion of recipients were detected by chance. The incidence of such strains might therefore be considerably higher, as indicated in a recent survey of *E. coli* from the Far East (Echeverria *et al.* 1978).

It has been suggested that antimicrobial agents might be useful in preventing travellers' diarrhoea caused by enterotoxigenic *E. coli* (Sack *et al.* 1978; DuPont *et al.* 1978). However, selective pressure of exposure to antibiotics would encourage transfer of genes for enterotoxin production and drug resistance together, whether these genes were carried on a single plasmid or on two separate co-transferable plasmids, thus leading to an increased incidence of drug-resistant, enterotoxigenic strains of *E. coli*. Since travellers' diarrhoea due to enterotoxigenic *E. coli* is usually a self-limiting disease, such unnecessary use of antibiotics is to be discouraged.

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