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Antibiotic-resistant *Escherichia coli* in market pigs in 1956–1979: the emergence of organisms with plasmid-borne trimethoprim resistance

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SUMMARY

Surveys conducted since 1956 on the antibiotic resistance of the *Escherichia coli* in faecal specimens from pigs entering Chelmsford Market have revealed that despite the implementation of the Swann Report in 1971 pigs are still an enormous reservoir of tetracycline-resistant *E. coli* with conjugative ability.

Increasingly large amounts of *E. coli* resistant to streptomycin and sulphonamides were found in specimens examined in recent years until in 1979 the amounts present approached those of tetracycline-resistant organisms.

E. coli resistant to chloramphenicol, ampicillin, neomycin, furazolidone or spectinomycin were present, usually in low concentration, in a considerable proportion of the specimens at each yearly examination but the concentration and incidence of these organisms showed no obvious sign of increasing with time. Much of this resistance, except to furazolidone, was of the transferable type.

Until 1979 the incidence of faecal specimens containing trimethoprim-resistant *E. coli* was very low. It increased significantly in that year, most of the resistance being plasmid-, or possibly transposon-determined.

The results of surveys performed in a Cambridgeshire market in 1978 and 1979, which showed that a high proportion of faecal specimens contained low concentrations of trimethoprim-resistant *E. coli*, in general resembled those of the corresponding Chelmsford surveys, suggesting that all the Chelmsford surveys may have accurately reflected the position in the national pig herd.

INTRODUCTION

After a period of some 17 years, the practice of feeding pigs continuously on diets containing tetracyclines was prohibited in the U.K. in 1971 because it had given rise to enormous populations of *Escherichia coli* organisms with transferable tetracycline resistance. The examination of faecal specimens from pigs brought to Chelmsford Market in 1956, 1970, 1972, 1973, 1974 and 1975 (Smith, 1973, 1975) suggested that in the 4 years of prohibition the amount of tetracycline-resistant *E. coli* in the pig population might have decreased slightly but that the incidence of pigs excreting these organisms had not (100% in 1975); the incidence of transferable tetracycline resistance, however, in 1974 and 1975 was only about half

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that found in 1970. The *E. coli* were also examined for resistance to other antibiotics, including those used in controlling disease in pigs, and the main change observed was the emergence of large populations of organisms that were resistant to streptomycin and sulphonamides. The present paper extends the observations to 1979 and, more particularly, records the emergence of *E. coli* populations with trimethoprim resistance, plasmid- or possibly transposon-determined. It also records the results of performing similar surveys on pigs brought to a large market in Cambridgeshire.

MATERIALS AND METHODS

Faecal specimens and their examination for antibiotic-resistant E. coli

At each survey, portions of individual faecal specimens from 100 pigs in different pens in the market were collected in sterile tubes; this method of sampling ensured that a high proportion of the pigs had come from different farms. A thick aqueous suspension of each specimen was spread evenly over the surface of two dried plates of MacConkey's medium and disks containing different antibiotics (Oxoid) were then applied at equal distances apart. The antibiotics were tetracycline (Tc), 50 μg; streptomycin (Sm), 25 μg; sulphafurazole (Su), 500 μg; chloramphenicol (Cm), 50 μg; ampicillin (Ap), 25 μg; neomycin (Nm), 30 μg; furazolidone (Fur), 15 μg; sodium nalidixate, 30 μg; polymixin, 300 units; spectinomycin (Spc), 25 μg; trimethoprim (Tp), 30 μg; and rifampicin, 25 μg, the actual antibiotics used depending on their availability. An exception to this procedure was the use before 1978 of DST agar (Oxoid) and a 1·25 μg disk for the trimethoprim tests. The plates were read after 24 h at 37 °C. The method of recording was subject to some variation, but, in general, it was as follows. All sensitive – when a broad zone containing no colonies resembling *E. coli* surrounded a disk. Nearly all sensitive – when a few colonies resembling *E. coli* were present in the inhibitory zone around a disk. Resistant and sensitive – when a disk was surrounded by a mainly confluent band of *E. coli* growth, less profuse than the mass of *E. coli* growth on the plate. All resistant – when growth of *E. coli* of uniform thickness took place up to the edge of the disk.

The methods of performing sensitivity tests on cultures and of determining the minimum inhibitory concentrations of antibiotics for these cultures have been described previously (Smith, 1976), as have the methods of identifying transferable and mobilizable resistance in *E. coli* (Smith, 1977); the concentration of trimethoprim or sulphafurazole in the selection plates of Sensitivity Test Agar used in these tests was 7·5 and 75 μg/ml respectively. In the transfer tests performed on the *E. coli* strains isolated in 1975 and thereafter, the mating mixtures of prospective donor strains and the prospective *E. coli* K12 recipient strain were held at room temperature for 24 h after the customary incubation at 37 °C to permit conjugation by thermo-sensitive plasmids.

RESULTS

The results of examining the faecal specimens from the Chelmsford and Cambridgeshire markets are summarized in Table 1. Throughout the years 1972–9 no decrease was observed in the incidence of tetracycline resistant (Tc^R) *E. coli* in the faecal specimens from the pigs brought to Chelmsford Market – of the 700 specimens examined in this period all except 17 were found to contain these organisms. The progressive decline in the amount of Tc^R *E. coli* in the specimens that occurred in 1972 and the few years thereafter was not maintained and at the 1979 examination all the *E. coli* in 40 % of the specimens appeared to be Tc^R. Following the great increase that occurred between 1956 and 1970 in the incidence and amount of streptomycin (Sm) and of sulphonamide (Su) resistance in the *E. coli* in the specimens, little change was observed in subsequent years until 1977 when an increase again commenced; by 1979 the incidence and amount of Sm^R and Su^R *E. coli* in the specimens resembled that of Tc^R *E. coli*. During all the examinations a close relationship was noted between the concentrations of Sm^R *E. coli* and Su^R *E. coli* in the individual specimens. At each yearly examination, spectinomycin-resistant (Spc^R) and ampicillin-resistant (Ap^R) *E. coli* were usually found in most specimens but only occasionally were they the dominant *E. coli* present. The incidence of chloramphenicol-resistant (Cm^R) *E. coli* was lower and there was no evidence of them becoming more common through the years; they were never present in high concentrations. Similar results were obtained for neomycin-resistant (Nm^R) and furazolidone-resistant (Fur^R) *E. coli* except that they were found less frequently than Cm^R organisms. Trimethoprim resistant (Tp^R) *E. coli* were not found in any of the specimens collected in 1972–5 but in 1977 and 1978 low concentrations were found in a few specimens. Their incidence increased sharply in 1979, the isolation rate of 42 % being increased to 59 % by culturing each faecal specimen on a whole plate of MacConkey's agar containing 400 μg/ml of trimethoprim. *E. coli* resistant to polymixin, sodium nalidixate or rifampicin were never isolated.

In general, the results for the Cambridgeshire specimens collected in 1978 and 1979 resembled those for the Chelmsford specimens. More Tp^R *E. coli*, though, were found in them in 1978 than in the corresponding Chelmsford specimens and the increase that occurred in 1979 was slighter than was the case in the Chelmsford specimens. Immediately after the 1979 Cambridgeshire market examination, a further 200 specimens from that market were examined; these comprised an equal number from fattened pigs, which would have been 6–8 months old, and from sows which would have been several years old. The percentage of specimens found to contain Tp^R *E. coli* by the customary disk test method was 30 for the fattened pigs and 49 for the sows. These figures increased to 44 and 78 respectively when the trimethoprim-containing plate method of examination was employed. The *E. coli* flora of the faeces of four of the sows appeared to consist entirely of Tp^R organisms.

The results of examining strains of *E. coli* derived from colonies that grew in the vicinity of the antibiotic-containing disks on the plates of MacConkey's agar employed for assessing the amount of antibiotic-resistant *E. coli* in the faecal specimens examined from the pigs brought to Chelmsford Market in 1972–9 and to

Table 1. *Antibiotic sensitivity of* E. coli *in pigs brought to Chelmsford and Cambridgeshire markets.*

Antibiotic	Year when specimens were collected	% of specimens in which the *E. coli* were: All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive
Tetracycline	1956	18	13	22	47
	1970	64	22	7	7
	1972	36	41	16	7
	1973	23	62	13	2
	1974	25	53	18	4
	1975	41	52	7	0
	1977	24	64	9	3
	1978	27 (49)	59 (37)	13 (9)	1 (5)
	1979	40 (35)	48 (46)	12 (18)	0 (1)
Streptomycin	1956	0	0	2	98
	1970	15	45	20	20
	1972	7	42	30	21
	1973	10	58	22	10
	1974	8	54	24	14
	1975	13	55	25	7
	1977	23	46	26	5
	1978	24 (14)	37 (38)	35 (22)	4 (26)
	1979	35 (27)	35 (38)	21 (22)	9 (13)
Sulphonamides	1956	2	2	4	92
	1970	12	32	18	38
	1972	5	41	29	25
	1973	10	58	22	10
	1974	13	48	21	18
	1975	14	51	26	9
	1977	23	48	24	5
	1978	24 (16)	38 (41)	34 (27)	4 (16)
	1979	35 (28)	35 (37)	21 (21)	9 (14)
Spectinomycin	1972	3	20	26	51
	1973	1	23	34	42
	1974	1	16	31	52
	1975	0	4	43	53
	1977	1	22	50	27
	1978	0 (2)	10 (15)	59 (17)	31 (66)
	1979	12 (2)	10 (24)	42 (20)	36 (54)
Ampicillin	1970	1	20	15	64
	1972	0	12	18	70
	1973	0	24	35	41
	1974	0	20	48	32
	1975	2	16	51	31
	1977	0	21	37	42
	1978	2 (5)	22 (20)	38 (30)	38 (45)
	1979	1 (4)	12 (22)	36 (24)	51 (50)
Chloramphenicol	1956	0	0	0	100
	1970	0	3	2	95

Table 1 (*cont.*)

Antibiotic	Year when specimens were collected	% of specimens in which the *E. coli* were: All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive
Chloramphenicol	1972	0	1	3	96
	1973	0	8	19	73
	1974	0	4	30	66
	1975	0	0	8	92
	1977	0	3	24	73
	1978	0 (0)	2 (4)	9 (15)	89 (81)
	1979	0 (0)	1 (5)	9 (14)	90 (81)
Neomycin	1970	1	1	5	93
	1972	0	1	1	98
	1973	0	5	7	88
	1974	1	0	7	92
	1975	1	2	5	92
	1977	0	0	3	97
	1978	0 (0)	1 (0)	1 (1)	98 (99)
	1979	0 (0)	0 (0)	1 (1)	99 (99)
Furazolidone	1970	1	3	4	92
	1972	1	1	6	92
	1973	0	4	13	83
	1974	0	0	2	98
	1975	0	0	1	99
	1977	0	2	17	81
	1978	0 (0)	2 (0)	12 (2)	86 (98)
	1979	1 (1)	3 (9)	8 (7)	88 (83)
Trimethoprim	1972	0	0	0	0
	1973	0	0	0	0
	1974	0	0	0	0
	1975	0	0	0	0
	1977	0	2	9	89
	1978	0 (0)	0 (5)	4 (17)	96 (78)
	1979	1 (1)	8 (11)	33 (17)	58 (71)
Polymixin and Sodium nalidixate	1972	0	0	0	100
	1973	0	0	0	100
	1974	0	0	0	100
	1975	0	0	0	100
	1976	0	0	0	100
	1977	0	0	0	100
	1978	0 (0)	0 (0)	0 (0)	100 (100)
	1979	0 (0)	0 (0)	0 (0)	100 (100)
Rifampicin	1977	0	0	0	100
	1978	0 (0)	0 (0)	0 (0)	100 (100)
	1979	0 (0)	0 (0)	0 (0)	100 (100)

The method of classifying the specimens according to their content of resistant and sensitive *E. coli* has been described in the text.

The results for the Cambridgeshire market are shown in parentheses.

Table 2. *Antibiotic resistance pattern of* E. coli *strains selected in 1972–9 because they were resistant to a particular antibiotic: the transferability of that resistance.*

Resistance selected	No. of strains examined that possessed it	Antiobiotic resistance pattern of the strains	% that were resistant to the following no. of antibiotics							% of strains that transferred the selected resistance
			1	2	3	4	5	6	7	
Tc	346	Tc (63), TcSmSu (10), TcSm (9), TcSmSuSpc (4), 12 others (14)	63	15	17	5	0	0	0	32
Sm	344	Sm (6), SmSu (44), TcSmSu (31) 23 others (19)	6	51	34	7	2	0	0	29
Spc	104	SmSpc (34), TcSmSpc (25), TcSmSuSpc (17) SmSuSpc (15), 17 others (9)	0	34	37	22	4	3	0	35
Cm	106	TcSmSuSpcApCm (30), TcSmSuSpcCm (24), TcSm SuSpcApCmFur (14), SmSuSpcCm (9), 10 others (13)	0	0	1	8	32	41	18	59
Ap	110	Ap (23), TcSmSuAp (20), TcAp (12), TcSmAp (11) SmSuAp (7), 14 others (27)	22	16	28	22	8	2	2	70
Nm	21	TcNm (33), Nm (29), SmSuNm (10), 4 others (28)	29	43	14	5	0	0	0	95
Fur	44	TcFur (32), TcSmSuSpcFur (16), Fur (11) TcSmSuSpcApCmFur (11), 6 others (30)	11	37	18	9	16	0	9	0

The figures in parentheses are the percentage of strains possessing the stated antibiotic resistance pattern.

the Cambridgeshire market in 1978 are summarized in Table 2; only one colony was picked from the vicinity of any one disk. There were no obvious differences between the results for the Chelmsford and the Cambridgeshire strains. None of the Chelmsford strains selected in this manner in 1956 as Tc^R were resistant to any of the other antibiotics against which they were tested. A substantial proportion of those isolated in subsequent years were resistant to other antibiotics, notably Sm and Su, but at all these examinations multiple resistance was never more common than resistance to Tc only; none of the strains were resistant to more than four antibiotics. Large antibiotic resistance patterns were also uncommon amongst strains selected as Sm^R. A feature of these strains was a high incidence of Su resistance. Of the 344 examined 90% were also Su^R. All the strains selected as Spc^R were, in addition, Sm^R. Large antibiotic resistance patterns were a feature of the strains selected as Cm^R, 91% being resistant to 5–7 antibiotics; all except one of them were resistant to Sm, Su and Spc. Although Tp resistance was found amongst strains selected as Tc^R, Sm^R, Spc^R or Ap^R it was commonest amongst the strains selected as Cm^R (13%). Moderately sized patterns were common amongst the strains selected as Ap^R, the commonest patterns being Ap^R and $SmSuSpcAp^R$.

The strains selected as Nm^R had the highest rate of resistance transfer followed by those selected as Ap^R, Cm^R, Tc^R, Spc^R and Sm^R. Fur resistance was not transferred from any strain. The rate of transferable Cm resistance recorded in Table 2 would have been considerably higher had it not been for our failure to demonstrate transfer from any ot 24 $TcSmSuSpcCm^R$ strains. The transfer rate from strains selected as Tc^R was highest in 1970 and 1972, 73% and 60% respectively of 30 strains examined on each occasion. The corresponding figures for 50 strains in 1974, 1975, 1977, 1978 and 1979 were 32, 36, 16, 36 and 32% respectively; for 50 strains from the Cambridgeshire market it was 30%. In 1974, the year in which the technique was modified to aid transfer by temperature-sensitive (ts) plasmids, the 50 Tc^R strains were also examined for degree of transfer at 37, 28, and 22 °C; one transferred less efficiently at 37 °C than at 28 and 22 °C and one failed to transfer at all at 37 °C. In a similar experiment with the 50 Tc^R strains obtained from the Chelmsford specimens in 1978, three failed to transfer at 37 °C; their plasmids belonged to incompatibility group H_2. Ts conjugative plasmids were not demonstrated in any of the 50 strains selected in 1974 as Sm^R. The resistance determinants of all of 15 strains with non-transferable SmSpc resistance were mobilized by conjugative plasmids F and/or I; the corresponding figure for 17 strains with non-transferable SmSu resistance was 14.

Because many more Tp^R *E. coli* were found in the Chelmsford specimens in 1979 than in preceding years and because substantial amounts were found in the Cambridgeshire specimens in that year and in 1978, Tp^R strains were studied in greater detail than were the strains selected as being resistant to other antibiotics. Details of their antibiotic resistance and, when Tp was used in the selection medium, of its transfer to *E. coli* K12 and of its mobilization in non-transferring strains by conjugative plasmid I is summarized in Table 3. Of the 209 Tp^R strains, 170 were resistant to Su, 159 to Sm and Spc and 127 to Sm, Su and Spc. Tp resistance was transferred from, or mobilized in, all except 10 strains, the commonest

Table 3. *Transfer of Tp resistance from pig* E. coli *strains; mobilization of the resistance in non-transferring strains by conjugative plasmid I*

Antibiotic resistance pattern of strains	No. of strains	No. that transferred Tp resistance	Antibiotic resistance pattern transferred	No. whose resistance was mobilized	Antibiotic resistance pattern mobilized
TcSmSuSpcTp	53	17	SmSpcTp (10), SmSuSpcTp (6), TcSmSpc Tp (1)	36	SmSpcTp (9), SmSuSpcTp (3), TcSmSpcTp (8)
SmSuSpcTp	45	17	SmSpcTp (10), SmSuSpcTp (3), SuTp (1), Tp (2), SmSuTp (1)	24	SmSpcTp (14), SmSuSpcTp (9), SuTp (1)
SuTp	19	3	SuTp (3)	14	SuTp (14)
SmSpcTp	17	4	SmSpcTp (4)	10	SmSpcTp (10)
TcSmSuSpcApTp	14	7	SmSpcTp (5), TcSmSpcTp (1), SmSpcApTp (1)	7	SmSpcTp (5), TcSmSpcTp (2)
TcSmSuApTp	9	4	TcSmSuApTp (3), SuApTp (1)	5	SuTp (5)
TcSuTp	8	6	SuTp (4), TcSuTp (1), Tp (1)	3	SuTp (2)
TcSmSpcTp	8	4	SmSpcTp (3), TcSmSpcTp (1)	4	SmSpcTp (4)
SmSuSpcNmTp	8	8	NmTp (4), SmSuSpcNmTp (3), SmSpcNmTp (1)	—	—
12 others	28	20	SmSpcTp (5), SuTp (3), NmTp (5), TcSmSuAp Tp (1), SmSpcTp (3), TcNmTp (1), Tp (1)	7	SmSpcTp (1), SuTp (6)
All 21	209	90	SmSpcTp (37), SuTp (11), SmSuSpcTp (9), NmTp (9), 8 others (24)	109	SmSpcTp (39), SuTp (42), SmSuSpcTp (12) TcSmSpcTp (10), TcSmSuSpcTp (2)

The figures in parentheses are the numbers of strains in which transfer or mobilization of the particular antibitoic resistance was detected when Tp was used in the selection medium.

antibiotic resistance pattern involved being SmSpcTp (from 76 strains) and SuTp (from 53 strains). Mobilization was also accomplished by conjugative plasmid F, generally less efficiently than by I. In the mobilization tests, recipient organisms were often found to have acquired only SmSpcTp or SmSu resistance from strains that were resistant to these four antibiotics and to any additional antibiotics. When Su was used in the selection medium instead of Tp, the strains that had donated SmSpcTp resistance were found to donate SmSu resistance and when Spc was used instead of Tp, the strains that had donated SuTp resistance were found to donate SmSpc resistance suggesting that these donor strains either contained an SmSpcTp and a SmSu plasmid or a SuTp and a SmSpc plasmid.

Four *E. coli* K12 strains that acquired SmSpcTp resistance from different donors in the I mobilization experiments were mated on several occasions with another *E. coli* K12 strain. All the recipient organisms tested from these matings had either acquired both I and the determinants for SmSpcTp resistance or neither. This suggestion of linkage between I and the resistance determinants in these four strains was confirmed by transduction experiments with phage P1. By contrast, linkage was not demonstrated between I and the SmSu determinants it had mobilized in five strains.

The minimum inhibitory concentration of trimethoprim for 209 Tp^R strains was > 1200 μg/ml; none was thymine requiring. All of 23 of the strains transferred their Tp resistance as efficiently at 37 °C as at 28 and 22 °C indicating that their transfer mechanism was not temperature-sensitive. None of the K12 strains that had acquired Tp resistance from 58 of the strains had also acquired determinants for resistance to mercury, arsenic or tellurite or for production of haemolysin or K88 antigen; seven had acquired determinants for colicine production.

DISCUSSION

The fact that the results of the two Cambridgeshire surveys, in general, resembled those of the corresponding Chelmsford surveys supports the view that the whole series of surveys carried out at Chelsmford may have fairly accurately reflected the position in the national pig herd. If this is so, then it appears from the results of the more recent surveys that the pig population of the U.K. will remain an enormous reservoir of Tc^R *E. coli* with conjugative ability for many years to come, a reminder of the profound and lasting ecological changes that can be brought about by feeding animals on diets containing antibiotics. Successive surveys have revealed little variation in the incidence and amount of Ap^R, Cm^R, Nm^R and Fur^R *E. coli* in the faecal specimens. Their emergence and persistence is the probable consequence of the veterinary use of antibiotics and if this does not increase greatly then the present state, almost of equilibrium, is likely to continue. The impact of veterinary antibiotic use has been quite different as far as $SmSu^R$ organisms are concerned in that they have become much more prevalent and in the 1979 survey they vied with Tc^R ones as the commonest antibiotic-resistant *E. coli* .Their emergence would be the consequence of the use of Sm or of Su because in most $SmSu^R$ strains examined the genes for resistance to both these

antibiotics appeared to be located on the same non-conjugative plasmid. The use of Sm, too, could have been responsible for the emergence of the $SmSpc^R$ organisms that were fairly frequently found in most of the surveys because their dual resistance was usually plasmid-borne and was probably due to synthesis of the adenylylating enzyme that inactivates both antibiotics as distinct from the phosphorylating enzyme that inactivates only streptomycin (Ozanne *et al.* 1969; Benveniste, Yamada & Davies, 1970), and which, presumably, was responsible for the streptomycin resistance of the SmSu plasmids.

Because Tp is always used therapeutically in combination with Su, the high incidence of Su resistance in the *E. coli* strains selected as Tp^R was anticipated. Although one plasmid determined both kinds of resistance in many of the strains, in most, two plasmids were probably responsible, the SmSu plasmid that was prevalent in the pig population well before it was exposed to mixtures of Tp and Su, and the SmSpcTp plasmids that were not. The latter may have derived from SmSpc plasmids that have been identified in pig *E. coli* ever since the 1972 survey, the first time spectinomycin resistance was assessed. These plasmids, like the SmSpcTp plasmids in the present investigation, had been found to integrate with conjugative plasmid I (Smith, 1977). Transposon 7, first described in human isolates of enterobacteria (Barth *et al.* 1976) and also reported in a *Salmonella typhimurium* strain of bovine origin (Richards *et al.* 1978), also codes for SmSpcTp resistance and it may be that this transposon is responsible for the SmSpcTp resistance in our pig strains. If so, the situation becomes potentially more serious because the results of the Chelmsford and Cambridgeshire surveys suggest that SmSpcTp resistance may already be widely dispersed throughout the U.K. pig population and if the present methods of Tp/Su use continue it could become sufficiently common to make its transfer to animal pathogens and its 'spill over' into the human community distinct possibilities; the former possibility has obviously not yet occurred to a substantial extent (West & White, 1979).

Apart from being given to sick domestic animals, mixtures of Tp and Su, like many other antibiotics, are given to groups of healthy animals in the hope of protecting them from contracting disease. This policy is much more likely to give rise to the emergence of antibiotic-resistant organisms than is the treatment of sick animals because the bacterial flora of a much larger number of animals will be exposed to the selection pressure provided by the antibiotics. The rationale of such a policy, in any case, is open to question, and, because of the great value of trimethoprim in human and animal medicine, the present investigation suggests that it should be discouraged.

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