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Escherichia coli resistant to tetracyclines and to other antibiotics in the faeces of U.K. chickens and pigs in 1980

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SUMMARY

A survey conducted in 1980, 9 years after the banning of the use of tetracyclines as feed additives in the U.K., indicated that table chickens and pigs were still a large reservoir of tetracycline-resistant *Escherichia coli*; the incidence of transferable tetracycline resistance was greater in chicken *E. coli* strains (68 %) than in pig *E. coli* strains (20 %). Large amounts of sulphonamide-resistant and of furazolidone-resistant *E. coli* were found in the faeces of chickens; *E. coli* resistant to both sulphonamides and streptomycin were common in the faeces of chickens and pigs. *E. coli* with transferable or mobilizable trimethoprim resistance were present in the faeces of most pigs and in the faeces of 10 % of chickens.

INTRODUCTION

After a period of some 17 years, the feeding of pigs and table poultry on diets containing tetracyclines was prohibited in the U.K. in 1971; the veterinary use of tetracyclines continued. During that time, the practice was even more common in chicken production than in pig production to the extent that in the several years immediately preceeding 1971 probably every broiler chicken in the U.K. was so fed. The reason for the prohibition was that the practice had given rise to enormous numbers of *Escherichia coli* organisms with transferable tetracycline resistance. The examination of faecal specimens from pigs brought to markets in Chelmsford and Cambridgeshire at different times between 1956 and 1979 indicated that the prohibition had had very little effect in bringing about a reduction in the incidence of these organisms in the pig population (Smith, 1973, 1975 and 1980). The faecal *E. coli* from the pigs were also examined for resistance to most of the commoner antibiotics available at the time each survey was carried out. Although there had been an increase in resistance to most of them, by far the greatest increase was in the incidence of organisms resistant to both streptomycin and sulphonamide. Another interesting feature of the survey was the emergence of trimethoprim resistance in 1977 and its rapidly increasing incidence in 1978 and 1979.

Because of the results of the pig studies, it seemed worthwhile surveying broiler chickens in 1980, particularly to see whether the ban on feeding diets containing tetracyclines had had any impact on their faecal *E. coli*, and comparing the results with a survey carried out at the same time on pigs; a survey on market chickens had been performed concurrently with the first survey on pigs in 1956. The results are reported in this paper. Pig faeces were collected at the Chelmsford market. Because market chickens would by no means be representative of the U.K. broiler chicken population, faecal samples for that survey were obtained by the much more time-consuming method of visiting a large number of poultry farms.

MATERIALS AND METHODS

The collection of 100 individual faecal specimens from pigs in Chelmsford market in a manner that ensured a high proportion came from different farms and the method of assessing the proportion of antibiotic-resistant *E. coli* in the specimens by smearing them evenly over the surface of plates of MacConkey's agar and applying disks containing different antibiotics has been described previously (Smith, 1980). In the present survey, trimethoprim-resistant *E. coli* were also detected by culturing each faecal specimen on a whole plate of MacConkey's agar containing 400 μg/ml of trimethoprim. The 100 individual chicken faecal specimens were collected from different houses on 40 poultry farms. The methods of performing sensitivity tests on cultures and of determining the minimum inhibitory concentrations of antibiotics for these cultures have been described previously, as have the methods of identifying transferable and mobilizable resistance (Smith, 1980).

RESULTS

The results of assessing the incidence and amount of antibiotic-resistant *E. coli* in the faecal samples collected from 100 chickens in 1980 are summarized in Table 1; those for the samples collected in the same year from 100 pigs and collected in 1956 from 100 chickens and 100 pigs are included for comparison. As in the case of the pigs, a high proportion of the *E. coli* in the faeces of the chickens examined in 1980, much higher than in those examined in 1956, were tetracycline-resistant (Tc^R); all the *E. coli* in about one-third of the specimens appeared to be Tc^R and in only a few of them were no Tc^R organisms detected. Again, as in the case of the pigs, very much more streptomycin (Sm) and sulphonamide (Su) resistance was present in the *E. coli* in the 1980 than in the 1956 chicken faecal specimens. The amount of Su resistance was greater than the amount of Sm resistance in the chicken *E. coli* whereas in the pig *E. coli* the amount of both forms of resistance was about the same; taken as a whole, the 1980 chicken specimens probably contained more Su^R *E. coli* organisms than Su^S *E. coli* organisms. The only other form of resistance that was common amongst the chicken *E. coli* was furazolidone (Fur) resistance; all the *E. coli* in about one quarter of the specimens appeared to be Fur^R and in only about one third of them were no Fur^R *E. coli* detected. The incidence and amount of *E. coli* organisms resistant to chloramphenicol (Cm), ampicillin (Ap), spectinomycin (Spc), neomycin (Nm) and trimethoprim (Tp) in the chicken specimens was low, lower, in general, than in the pig specimens; even by means of the more sensitive plate method, *E. coli* possessing resistance to trimethoprim (Tp) were detected in only 10% of the chicken specimens whereas such *E. coli* were detected in 69% of the pig specimens.

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

Table 1. *Antibiotic sensitivity of* E. coli *in faeces of* 100 *chickens and pigs in* 1956 *and* 1980

		% of faecal specimens from							
		chickens in which the *E. coli* were				pigs in which the *E. coli* were			
Antibiotic	Year when specimens were collected	All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive	All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive
Tetracycline	1956	5	13	14	68	18	13	22	47
	1980	32	49	11	8	40	49	8	3
Streptomycin	1956	0	0	0	100	0	0	2	98
	1980	10	30	36	24	27	54	15	4
Sulphonamides	1956	16	5	6	73	2	2	4	92
	1980	64	27	5	4	34	49	14	3
Chloramphenicol	1956	0	1	0	99	0	0	0	100
	1980	0	0	8	92	0	0	9	91
Ampicillin	1980	1	4	24	71	6	31	37	26
Furazolidone	1980	25	14	26	35	2	6	17	75
Spectinomycin	1980	0	3	28	69	4	20	40	36
Neomycin	1980	0	0	2	98	0	1	6	93
Trimethoprim	1980	0(0)	0(0)	4(10)	96(90)	1(1)	2(2)	40(66)	57(31)
Polymyxin, sodium nalidixate and rifampicin	1980	0	0	0	100	0	0	0	100

The results of the plate tests for trimethoprim resistance are shown in parenthesis.

Table 2. *Antibiotic resistance patterns in* E. coli *strains selected in the* 1980 *chicken and pig surveys because they were resistant to a particular antibiotic: the transferability of that resistance*

Resistance selected	Strains examined: Source	Strains examined: No. possessing the resistance	Antibiotic resistance patterns of the strains	% that were resistant to the following no. of antibiotics: 1	2	3	4	5	6	7	% of strains that transferred the selected resistance
Tc	Chicken	50	Tc(36), TcSu(14), TcSmSu(14), TcSmFur(12), 3 others (24)	36	26	26	12	0	0	0	68
	Pig	50	Tc(26), TcSm(18), TcSmSu(12), TcSmSuSpc(10), 10 others (34)	26	26	20	24	4	0	0	20
Sm	Chicken	50	Sm(12), SmSu(36), TcSmSu(32), TcSm(8), 4 others (12)	12	42	42	4	0	0	0	22
	Pig	50	Sm(8), SmSu(30), TcSmSu(20), TcSmSuAp(8), 12 others (34)	8	36	30	18	8	0	0	30
Su	Chicken	50	Su(48), SuFur(14), TcSu(10), TcSuFur(8), 5 others (20)	48	30	18	4	0	0	0	24
Cm	Chicken	8	TcSmSuSpcCm(50), TcSmSuSpcCmFur(50)	0	0	0	0	50	50	0	75
	Pig	9	TcSmSuCmApSpc(45), TcSmCm(11), CmFurTp(11), 3 others (33)	0	0	22	11	11	45	11	78
Ap	Chicken	20	Ap(5), TcAp(20), ApFur(15), SmSuAp(10), 8 others (50)	5	45	20	15	15	0	0	55
	Pig	20	Ap(10), TcSmSuApSpc(25), TcSmSuAp(15), TcAp(10), 7 others (40)	10	15	30	5	25	5	0	65
Fur	Chicken	20	Fur(15), SuFur(25), TcFur(15), TcSmSuFur(15), 3 others (30)	15	45	25	15	0	0	0	0
	Pig	20	Fur(10), TcSmSuFur(20), TcSmFur(15), TcSmSuSpcFur(15), 7 others (40)	10	15	20	30	25	0	0	0

Spc	Chicken	20	SmSuSpc(45), SmSuSpcFur(25), TcSmSuCmSpc(15), 2 others (15)	0	5	45	35	15	0	0	35
	Pig	20	SmSpc(25), TcSmSuSpc(25), SmSuSpc(20), TcSmSpc(15), 3 others (15)	0	25	40	25	5	5	0	20
Nm	Chicken	2	TcSmNm(50), TcSmSuFurNm(50)	0	0	50	0	50	0	0	100
	Pig	7	TcSmSuNm(29), TcNm(14), TcSmSuFurNm(14), 4 others (43)	0	14	14	43	29	0	0	100
Tp	Chicken	10	Tp(20), TcTp(20), SuTp(20), 5 others (40)	20	40	20	20	0	0	0	70
	Pig	69	Tp(3), SuTp(26), SmSuSpcTp(16), TcSmSuSpcTp(15), 13 others (40)	3	26	17	22	25	6	1	30

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

employed for assessing the amount of antibiotic-resistant *E. coli* in the chicken and pig specimens collected in 1980 are summarized in Table 2; only one colony was picked from the vicinity of any one disk. Small antibiotic resistance patterns were a feature of the chicken *E. coli* selected as Tc^R, Sm^R, Su^R, Fur^R or Tp^R; nearly half of the strains selected as Su^R were Su^R only and over one-third of those selected as Tc^R were Tc^R only. All the chicken strains selected as Spc^R were also Sm^R and SmSu resistance was common amongst these strains as it was amongst those selected for resistance to other antibiotics. Large antibiotic resistance patterns were much more common amongst the strains selected as Cm^R. Allowing for the greater amount of Su and Fur resistance to the chicken strains, the antibiotic resistance patterns in the pig strains were broadly similar to those in the chicken strains.

Apart from the much higher rate of transferable Tc resistance in the chicken Tc^R strains (68 %) than in the pig Tc^R strains (20 %), the incidence of the different kinds of transferable resistance was similar in the chicken and pig strains; the difference in the case of trimethoprim resistance probably being a reflection of the small number of chicken strains examined that possessed this kind of resistance. Transferable resistance was highest in the case of Nm and Cm resistance, lowest in the case of Su and Sm resistance and non-demonstrable in the case of Fur resistance. Su resistance was transferred from only 4 of 24 chicken strains that were Su^R only; it was mobilized by conjugative plasmid I from one of six of these strains. It was transferred with Sm resistance from one of 18 $SmSu^R$ chicken strains but from two of four of them Sm and Su were mobilized by I. By means of I it was also possible to mobilize the resistance in all three chicken strains, and 39 of the 48 pig strains, selected as Tp^R which did not transfer their resistance direct.

DISCUSSION

Because little significant change was found in the amount of tetracycline-resistant *E. coli* in the faeces of U.K. pigs since the feeding of diets containing tetracyclines to pigs and table poultry was banned in 1971, it was concluded that the pig population in the U.K. would probably remain an enormous reservoir of Tc^R *E. coli* with conjugative ability for many years to come (Smith, 1980). The present survey on chickens, the first of its kind since the ban, indicates that at the present time the chicken population might be an even larger reservoir, particularly as the incidence of transferable resistance in the Tc^R *E. coli* examined from the chickens (68 %) was more than three times higher than that in the pig strains (20 %). In only two of the pig surveys conducted by Smith, those in 1970 and 1972, the years immediately preceeding and following the introduction of the ban, did the incidence of transferable Tc resistance approach that found in the present chicken survey; the percentage figures for 1970, 1972, 1974, 1975, 1977, 1978 and 1979 were 73, 60, 32, 36, 16, 36 and 32 respectively.

The amount of Su^R *E. coli* in the chicken specimens was even greater than the amount of Tc^R *E. coli* but the incidence of transferable Su resistance was much lower than the incidence of transferable Tc resistance. It is difficult to avoid opining that the enormous amount of Su^R *E. coli* that the present survey suggests exists in the U.K. chicken population is neither a relic of the time when sulphonamides, such as sulphadimidine, which is fairly active against *E. coli*, were commonly used in the flock treatment of outbreaks of coccidiosis nor the long term consequence of the later policy of preventing coccidiosis by feeding chickens continuously on diets containing low concentrations of sulphaquinoxaline, a sulphonamide weakly active against *E. coli*. In comparatively short-term studies, such diets have not been found to influence the Su^R *E. coli* content of the alimentary tract of chickens (Guinée & Kruyt, 1975).

Because the genes for streptomycin resistance and sulphonamide resistance commonly exist on the same plasmid, it is probable that sulphonamide usage is largely responsible for the considerable amount of Sm resistance found in the chicken *E. coli*, streptomycin rarely being given to chickens. The explanation for the large amount of $SmSu^R$ *E. coli* in the pig population is different in that most of it would be the consequence of the veterinary use of either of the two antibiotics in controlling bacterial disease. This type of useage would probably account for most of the other kinds of resistance encountered, including that to furazolidone, expectedly higher in the chicken than in the pig strains because of its greater application in chickens, and to trimethoprim, expectedly higher in the pig strains because of its greater application in pigs. Whereas the high incidence of Fur resistance is only of importance in the case of those strains that are pathogenic because Fur resistance is non-transferable, the presence of Tp^R *E. coli* in a high proportion of the pig samples that were examined is of greater concern because of the transferable nature of Tp resistance and because trimethoprim is a valuable antibiotic for treating human beings and animals.

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