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The persistence of drug resistant *Escherichia coli* in the intestinal flora of healthy broiler chicks

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SUMMARY

Antibacterial drugs (oxytetracycline, streptomycin and sulphonamides) were included in the drinking water of healthy broiler chicks from the sixth to the twentieth day of life to select a resistant gut flora. On the twenty-first day the birds were divided into three groups and reared in separate rooms until 100 days of age. One group was housed in cages with wire floors while the others were reared on litter. Faeces from adult hens were added regularly to the litter of one of these groups to determine its effect on the gut flora of the chicks.

The ecology of *Escherichia coli* was studied using O-serotyping, biotyping and antibacterial drug resistogram typing. The proportion of *E. coli* in the dominant faecal flora resistant to two to four antibacterial drugs increased with time to reach a peak several days after the drugs were withdrawn. Thereafter, the level of drug resistance in the *E. coli* declined equally in all three groups. The majority of organisms with multiple resistance were derived from biotypes of O-serotypes initially resistant to only one drug and were identified before the drugs were administered. The decline in the level of resistance in the dominant faecal flora after the fourth week was due to the appearance of either new O-serotypes or new biotypes of O-serotypes previously shown to be multiply resistant, and which were either sensitive or resistant to only one drug. It is probable that these new strains were derived from the food since several O-serotypes appeared simultaneously in all three groups of birds.

INTRODUCTION

It is impossible to avoid some degree of bacterial contamination of the carcasses of poultry killed and packed under commercial conditions and *Escherichia coli* derived from carcasses may colonize the intestinal tract of people who prepare the uncooked meat in the kitchen (Linton *et al.* 1977). This observation indicates

that there is a potential risk of drug resistant *E. coli* of animal origin being introduced to the human population. This risk can be reduced, but not eliminated, by feeding strains of *E. coli*, which are both good colonizers and sensitive to antibacterial drugs, to broiler chicks prior to slaughter (Linton *et al.* 1978).

It has also been demonstrated that the exposure of young chicks to adult gut flora reduced the incidence of salmonella excretion in experimentally infected animals (Rantala & Nurmi, 1973; Lloyd, Cumming & Kent, 1977; Snoeyenbos, Weinack & Smyser, 1977). The purpose of this investigation was to assess whether exposure of broiler chicks to adult hen faeces had any influence on the persistence of resistant *E. coli* in the intestinal tract. The ecology of *E. coli* in the chicken gut was monitored at the same time using serotyping and biotyping to identify strains.

MATERIALS AND METHODS

Broiler chicks

Day-old Ross chicks of both sexes purchased from a commercial hatchery were housed on wood shavings initially in one room, with cement-rendered floors and walls until the end of the brooding period at 20 days of age. The available floor area for the birds was 3·1 m by 2·1 m.

Oxytetracycline (100 p.p.m.), streptomycin (12·5 p.p.m.) and a mixture of sulphadiazine, sulphamerazine and sulphapyridine (65 p.p.m.) were administered in the drinking water from the 6th to the 20th day to select a predominantly resistant gut flora.

On the 21st day the birds were divided into three groups. Group A (10 birds) was housed in three cages with wire floors. Groups B and C (40 birds each) were reared on the floor with wood shavings as litter. Group B remained in the room used for the brooding period on fresh wood shavings plus half of the litter already present. Group C was moved, together with half of the litter from the brooding room, into an identically appointed room in the same building. About 2·5 kg of faeces obtained from adult laying hens kept in battery cages was sprinkled weekly over the litter of the chicks in Group C.

Commercially compounded poultry feeds were used throughout the experiment. These contained a growth promoter (nitrovin) and coccidiostat (dinitroorthotolumide). 'Starter' crumbs were fed for the first 28 days and 'finisher' pellets for the remainder of the experiment which was concluded when the birds were 100 days of age.

Bacteriological investigations

Collection of samples

Cloacal swabs were taken from the chicks on 17 occasions during the experimental period on days 1, 4, 8, 12, 18, 21, 25, 28, 35, 42, 49, 56, 63, 70, 88, 95 and 100. Ten birds, randomly selected, were swabbed on each occasion up to the 18th day; thereafter, five birds were examined at random in each of the three groups. In addition, swabs of the caecal and duodenal contents were obtained from five birds slaughtered from Group B on days 63, 70 and 100 and from Group C on days 70 and 100.

Table 1. *The distribution, according to resistance pattern of* E. coli *isolated from chicks in Groups A, B and C according to age*

			Group A*		Group B		Group C	
Age in days	1–5	6–18	21–28	35–100	21–28	35–100	21–28	35–100
Resistance pattern†								
Sensitive	—	1 (0·7)	4 (5·6)	81 (37·0)	3 (4·0)	61 (27·4)	7 (9·8)	59 (26·3)
S – – –	—	—	—			1 (0·4)	—	—
– Su – –	88 (88)	8 (5·4)		69 (31·5)	1 (1·3)	72 (32·3)		89 (39·7)
– – T –	4 (4)	17 (11·5)	4 (5·6)	5 (2·3)	1 (1·3)	30 (13·5)	2 (2·8)	28 (12·5)
S Su – –	3 (3)	21 (14·2)	1 (1·4)	3 (1·4)	3 (4·0)	3 (1·3)	—	—
S – T –	—	7 (4·7)	3 (4·2)	1 (0·4)			—	2 (0·9)
– Su T –	5 (5)	26 (17·6)	6 (8·5)	20 (9·1)	1 (1·3)	13 (5·8)	9 (12·7)	13 (5·8)
S Su T –	—	68 (45·9)	53 (74·6)	40 (18·3)	56 (74·7)	42 (18·8)	42 (59·2)	33 (14·7)
S Su T A	—	—	—	—	10 (13·3)	1 (0·4)	11 (15·5)	—
Total	100	148	71	219	75	223	71	224

* The birds were reared as one group until day 21. Antibiotics were administered in the drinking water between days 6 and 20. Birds in group A were then reared on wire; in group B on wood shavings and in group C on wood shavings plus adult hen faeces.

† S = streptomycin, Su = sulphafurazole, T = tetracycline and A = ampicillin.

Cloacal swabs were also collected from five of the laying hens supplying adult faeces, twice before the start of the experiment and then on days 21, 88, 95 and 100.

Isolation and identification of E. coli

The swabs were always processed on the day of collection by plating directly onto bile lactose agar without salt (BLA, Oxoid CM7b). The plates were incubated overnight at 37 °C. Five lactose-fermenting colonies, typical of *E. coli* were subcultured from each plate onto BLA for subsequent testing. All isolates were identified as *E. coli* on the basis of a positive indole and Eijkman test.

Serotyping and biotyping

All isolates were serotyped by a microagglutination technique using 157 grouping sera (Hartley *et al.* 1975; Howe & Linton, 1976) and biotyped on the basis of their ability to produce acid from adonitol, dulcitol, raffinose, rhamnose and sorbose (Hinton, Allen & Linton, 1982).

Determination of the in vitro *resistance to antibacterial drugs*

The antibacterial drug resistance pattern of all isolates was determined by a disk diffusion method (Linton, Howe & Osborne, 1975). The disks used included ampicillin (25 μg) (A), chloramphenicol (50 μg), kanamycin (30 μg), streptomycin (25 μg) (S), sulphafurazole (500 μg) (Su) and tetracycline (50 μg) (T).

RESULTS

The first five days of life

The birds were sampled on the first and fourth day of life prior to the administration of the antibacterial drugs in the drinking water. Of the 100 *E. coli* examined 92 were resistant to one drug (Su or T) and the remainder to two (Table 1). Five O-typable serotypes were identified namely O 8 (4 isolates), O 9 (71), O 32 (10), O 123 (7) and O 131 (4) (Table 2).

Days 6–18

The birds were sampled three times during the period the drugs were included in the drinking water and a progressive increase was demonstrated in the proportion of strains of *E. coli* showing multiple drug resistance. Taking the period as a whole only 16·9 % were resistant to one antibiotic, 36·5 % were resistant to two and the remaining 45·9 % were resistant to three antibiotics (Table 1). One hundred and fifteen (82 %) of the 140 O-typable *E. coli* isolated during this period were of the same O-serotypes as were isolated on the first two sampling occasions (Table 2). The biotypes within these serotypes were the same as those identified prior to the administration of the drugs in 110 (97 %) of the 113 isolates examined. This indicates that many of the resistant *E. coli* were selected from biotypes of O-serotypes present in the dominant faecal flora by the fourth day of life.

Days 21–100

The distribution of *E. coli* isolated from the three groups of birds according to O-serotype and resistance pattern is set out in Tables 1 and 2. The proportion of strains resistant to three or four drugs peaked in samples collected after the withdrawal of the drugs from the drinking water, on either the 21st or the 25th day. Taking the period 21–28 days of age 74·6 % of the isolates in group A, 88 % of isolates in group B and 74·7 % of the isolates in group C were resistant to three or four antibiotics. Thereafter, there was a fall, with time, in the proportion of isolates resistant to three or four drugs in all three groups with a corresponding increase in the proportion of strains that were either fully sensitive or resistant to one drug.

A total of 27 'new' O-serotypes appeared in the birds between the 21st and the 100th day (19 in Group A and 12 each in Groups B and C) (Table 2). When the three groups were considered together the number of 'new' O-serotypes identified at a sampling varied from none on 16 occasions to one on seven, two on nine, three on two, four on one and eight on one respectively. The majority of these serotypes were transient members of the dominant flora; fifteen O-serotypes (O 5, 13, 14, 19, 20, 49, 85, 86, 106, 111, 126, 137, 143, 148 and 163) were represented by only one or two isolates while three O-serotypes (O 7, 11 and 93) were detected in one group only. Of the remaining nine O-serotypes two (O 1 and 34) were recorded first in two groups on the same sampling occasion while three (O 2, 39 and 77) appeared in all three groups at either the same sampling occasion or at two adjacent samplings. This observation suggests a common source for these O-serotypes and

Table 2. *The distribution of* E. coli *amongst O-serotypes isolated from chicks at different ages*

Age in days...	1–5	6–18	21–100		
			Group A (wire)	Group B (shavings)	Group C (shavings and faeces)
O-serotype					
8	4	3	7	15	26
9	71	25	21	21	24
32	10	23	69	25	6
123	7	53	9	18	24
131	4	11	9	2	1
88	—	8	—	14	19
100	—	13	—	—	—
113	—	4	1	—	—
1	—	—	—	7	11
2	—	—	13	5	2
5	—	—	—	1	—
7	—	—	—	5	—
11	—	—	—	7	—
13	—	—	1	—	—
14	—	—	1	—	—
19	—	—	1	—	1
20	—	—	1	—	—
34	—	—	2	—	4
39	—	—	4	1	2
49	—	—	1	—	—
77	—	—	54	70	87
85	—	—	1	—	—
86	—	—	—	2	—
93	—	—	11	—	—
102	—	—	7	8	5
106	—	—	—	1	—
111	—	—	2	—	—
126	—	—	1	—	—
134	—	—	6	3	1
137	—	—	—	—	1
140	—	—	29	—	2
141	—	—	—	10	7
143	—	—	1	—	1
148	—	—	2	—	—
163	—	—	1	—	—
NT*	4	8	35	83	71
Total	100	148	290	298	295

* NT = not typable.

since the birds were housed in separate rooms within the same building it is probable that they were derived from the food.

Serotype O 77, the most successful colonizer of the 'new' O-serotypes, was identified first on the 35th day and 211 isolations were made from birds in the three groups. Of these 186 (88%) were biotype 6. Twenty-four of the other 25 isolates

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Table 3. *The distribution, according to resistance pattern of* E. coli *isolated from chicks in groups A, B and C in the periods* 21–28 *days and* 35–100 *days.*

(A) O-serotypes first identified between days 1 and 18 (see Table 2)

Resistance pattern	Age of birds (days) when *E. coli* isolated		Total (%)
	21–28	35–100	
Sensitive	2	22	24 (7·7)
– Su – –	1	16	17 (5·5)
– – T –	4	22	26 (8·4)
S Su – –	3	6	9 (2·9)
S – T –	—	2	2 (0·6)
– Su T –	10	34	44 (14·1)
S Su T –	94	75	169 (54·3)
S Su T A	20	—	20 (6·4)
Total	134	177	311

(B) O-serotypes identified first between days 21 and 100 (see Table 2)

Sensitive	8	127	135 (35·2)
– Su –	—	206	206 (53·8)
– – T	2	18	20 (5·2)
– Su T	—	2	2 (0·5)
S Su T	15	5	20 (5·2)
Total	25	358	383

were distributed in three biotypes (nos. 1, 15 and 16) each of which differed from biotype 6 by only one test result. All but one of these new biotypes were isolated 18 days or more after the first appearance of serotype O 77. Variation in biotypes within clones of *E. coli* isolated from human patients over a period of time has been recorded by Crichton & Old (1979) and they suggest that the explanation for these changes may be due to the acquisition or loss of a plasmid or phage. Only relatively few *E. coli* (six to 20) were represented by serotypes O 1, 2, 34 and 39 and an analysis of the biotypes within these O-serotypes proved inconclusive.

The *E. coli* isolated between the 21st and 100th day could be divided into two categories. The first comprised those amongst the eight O-serotypes identified before the 20th day of life ('initial' O-serotypes) and the second, the 27 O-serotypes recorded only later ('new' O-serotypes). The sensitivity patterns of the *E. coli* in these two categories were compared (Table 3); 244 (78·5%) of the 311 *E. coli* comprising the 'initial' O-serotypes were resistant to two to four drugs while 60 of the 67 isolates resistant to none or one drug were isolated between the 35th and the 100th day. On the other hand only 22 (6%) of the 'new' O-serotypes were resistant to two to four drugs and 15 of these were isolated between the 21st and 28th day during which time the peak in the proportion of multiply resistant strains was recorded. The difference in the distribution of resistance patterns in the 'initial' and 'new' O-serotypes was statistically significant ($P = < 0{\cdot}001$).

The distribution of biotypes amongst the 311 isolates comprising the seven 'initial' O-serotypes was analysed. The majority of isolates (234) were amongst biotypes identified before the 20th day. Of the 77 isolates within biotypes identified

Table 4. *The distribution of* E. coli *isolated from the duodenum, caecum and cloaca of birds from Groups B and C according to the number of R factors that the* E. coli *carried*

No. of R factors	No. (%) of *E. coli* isolated from		
	Duodenum	Caecum	Cloaca
0	29 (52·7)	51 (33·6)	37 (29·6)
1	6 (10·9)	51 (33·6)	76 (60·8)
2	12 (21·8)	44 (28·9)	5 (4·0)
3	7 (12·7)	5 (3·3)	7 (5·6)
4	1 (1·8)	1 (0·7)	—
Total	55	152	125

Table 5. *The distribution of the O-serotypes of* E. coli *isolated from the duodenum, caecum and cloaca of birds in Groups B and C*

	No. (%) of *E. coli* isolated from		
	Duodenum	Caecum	Cloaca
O-serotypes identified first before the 20th day of life (see Table 2)	13 (36)	19 (23)	10 (10)
O-serotypes identified first after the 20th day of life (see Table 2)	23 (64)	63 (77)	86 (90)
Total	36	82	96

for the first time after the 20th day, 33 were resistant to two to four drugs and 30 of these were amongst biotypes identified first between the 21st and 28th day. Conversely all 44 of the 'new' biotypes that were sensitive or resistant to one drug were identified first between the 35th and the 100th day of the rearing period. This suggests that these 'new' biotypes were new colonizing strains of *E. coli*, although they were of the same serotype as those initially isolated, and were probably derived from the food, like the other *E. coli* O-serotypes that were resistant to none or one drug.

The proportion of *E. coli* strains that were non-typable by the 157 O-grouping sera was low (5%) in birds aged under 3 weeks. The comparable proportions for birds reared on wire (Group A) and on litter (Groups B and C) were 12% and 26% respectively. In each group the proportion was significantly different from the other two ($P = < 0{\cdot}005$). The reason for this remains obscure.

A total of 332 *E. coli* were isolated from the caecum, duodenum and cloaca of 25 birds from Groups B and C slaughtered on the 63rd, 70th and 100th day of life. Seventy (34%) of the 207 *E. coli* isolated from the duodenum and caecum were resistant to two to four drugs as compared to 12 (9·6%) of 125 cultured from the cloaca (Table 4). This difference is significant ($P = < 0{\cdot}001$). The distribution of O-serotypes amongst the 214 typable strains was analysed according to whether the O-serotype was identified before or after the 20th day of life (Table 5) and this

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Table 6. *The O-serotypes of* E. coli *isolated from both the hens and the chicks*

		Nos. of *E. coli* isolated from				
			Chicks			
O-serotype	Biotype*	Hens	Aged 1–18 days	Group A	Group B	Group C†
2	6	—	—	—	—	1
	15	14	—	12	6	1
	22	—	—	1	—	—
8	6	30	7	—	1	1
	15	5	1	—	3	7
21	6	1	—	—	—	—
39	5	—	—	—	—	1
	13	—	—	—	—	1
	29	1	—	4	—	—
	30	—	—	—	1	—
134	6	23	—	6	3	1

* Based on the fermentation of adonitol, dulcitol, raffinose, rhamnose and sorbose (Hinton, Allen & Linton, 1982).

† The chicks in Group C were reared on wood shavings to which adult hens' faeces were added each week from the twenty-first day of life.

indicates that 32 (27 %) of 118 *E. coli* isolated from the duodenum and caecum were amongst O-serotypes identified before day 20 as compared to 10 (10·4 %) of 96 cloacal strains. This difference in the distribution of the O-serotypes is significant ($P = < 0{\cdot}01$) and it helps explain why the level of resistance was lower in the *E. coli* isolated from the cloaca. It also poses the question that there may be quantitative differences in the distribution of O-serotypes at different levels of the intestinal tract in healthy chicks, although proof of this cannot be determined from the available data.

The hens

Cloacal swabs were obtained from the hens on six occasions. The sensitivity pattern of 180 *E. coli* isolates was assessed; 129 were fully sensitive, 50 were resistant to Su and one to SSu. The O-serotype and the biotype of the 90 *E. coli* isolated during the rearing period of the chicks were determined and the results are set out in Table 6, together with data obtained for the comparable O-serotypes identified in the chicks before or after the 20th day of life. The *E. coli* in the dominant flora of the hens appeared to be relatively stable with 72 of the 74 O-typable strains being distributed amongst three O-serotypes (O 2, 8 and 134). Only 10 (3·4 %) of the 295 *E. coli* isolated from the chicks in Group C, which were exposed to adult hens' faeces each week, were the same O-serotype and biotype as those identified in the hens while some of the biotypes of serotypes O 2, 8 and 134 were also identified in chicks in the other two groups, e.g. serotype O 2 biotype 15; O 8 biotypes 6 and 15 and O 134 biotype 6.

DISCUSSION

The principal conclusions to be drawn from this study are as follow. The administration of antibacterial drugs in the drinking water led to a gradual development of multiple resistance amongst the chicks' dominant *E. coli* flora. The majority of the organisms with multiple resistance were probably selected from biotypes of O-serotypes present in the faecal flora before the drugs were given and which were resistant to only one drug. These strains may have been present in the minority flora of the birds before the drugs were administered since a few strains resistant to SSu and SuT were identified on the fourth day. However, this issue cannot be resolved from the available data since BLA supplemented with the appropriate antibacterial drugs was not used for primary isolation. The peak in the level of resistance occurred several days after withdrawal of the drugs. O-serotypes not recognized during the period of drug administration, but which were multiply resistant, appeared for the first time between the time that the drugs were withdrawn and the peak in drug resistance was reached. A fall in the level of drug resistance in the *E. coli* occurred with time and this was not influenced either by the addition of hens' faeces to the litter or by the rearing of the birds in cages with wire floors. The reason why the O-serotypes in the hens, all of which were probably good colonizers since the *E. coli* population in the hens appeared stable, did not become established in the chicks' intestines is not clear. There may have been inadequate dispersal of the faecal *E. coli* into the surrounding litter since both faeces and the litter were relatively dry at all times. The change in resistance patterns was due principally to the displacement of the multiply resistant *E. coli* by strains which were either sensitive or resistant to one drug and not to a loss of R determinants from biotypes of O-serotypes previously shown to be multiply resistant. Several of the O-serotypes resistant to none or one drug appeared simultaneously in all three groups of birds and, as these were housed in separate rooms, it is suggested that these *E. coli* were derived from the food.

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