RESEARCH NOTES

Transferable Drug Resistance in *Escherichia coli* Isolated from Antibiotic-Fed Chickens

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ABSTRACT The isolation and transferable drug resistance of *Escherichia coli* from the feces of chickens after oral administration of tetracylcine, chloramphenicol, rifampicin, and amoxicillin were studied. Each antibiotic was administered at two different dosages to four groups of 12 chickens. Treatment was carried out for 3 weeks. Feces were taken weekly and bacteriological examinations performed. *E. coli* biotypes were identified by fermentation and enzymatic reaction patterns. Antibiotic sensitivity tests were performed on all *E. coli* isolates.

Rapid appearance of *E. coli* biotypes showing drug-resistance to each antibiotic was observed as soon as 1 week after treatment. Resistance was not detectable a few days after interruption of antibiotic administration.

All *E. coli* strains showing drug resistance to the antibiotic under examination were studied to observe their capacity to transfer antibiotic resistance to *E. coli* K 12 E 711 F-strain. A high percentage of resistant *E. coli* strains transferred their antibiotic resistance to *E. coli* K 12. Transferable drug resistance was demonstrated mainly in tetracycline resistant *E. coli.*
(Key words: E. coli, chickens, drug - resistance)

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INTRODUCTION

The use of antibiotics in veterinary practice and the addition of antibiotics to animal feed are considered the most important causes of the appearance of drug resistance in bacteria of animal origin (Smith, 1968; Gardner, 1978). Recently the Food and Drug Administration (FDA) has emphasized the problem that antibiotic-fed animals frequently present resistant strains of Enterobacteriaceae that increase the spread of drug resistance to man. In fact, these resistant strains could colonize, even if transiently, in the human gut and/or transfer their R-factors to the intestinal flora of man. A great likeness between the plasmids of Enterobacteriaceae isolated in animal and man has been observed. Homologies of molecular weight and sequences of DNA bases of the regions codifying the resistance to different antibiotics have been observed in bacteria of human and animal origin (Anderson *et al.*, 1975; Fein *et al.*, 1974; Levy *et al.*, 1976). This study was to observe the intestinal *Escherichia coli* flora changes and the eventual appearance of transferable drug resistance in the chicken after antibiotic administration.

MATERIALS AND METHODS

Test Animal. Changes in antibiotic sensitivity of *E. coli* isolates in 3-month-old chickens from the Aviary Center of Portici, University of Naples, were examined after oral administration of the following four antibiotics: chlortetracycline (CTA), chloramphenicol (CAF), rifampicin (RN), amoxicillin (AMX).

All animals under examination had no previous antibiotic administration. Each antibiotic was administered to 12 chickens subdivided into three groups. One group was considered as control. Each group of 4 chickens was kept in an individual hen coop. The CTA and CAF were administered to each group of chickens at 110 and 440 mg/kg of feed; RN at 40 and 80 mg/kg of feed; and AMX at 200 and 400 mg/kg of feed.

Sampling Procedures. Pools of feces (6 hr accumulation) were collected from each group of 4 chickens with a sterile spatula before treatment, after 1, 2, and 3 weeks of treatment, and at 3 and 7 days after the interruption of treatment. The hen coops and water jars were sterilized before each experiment.

Identification of E. coli. Bacteriological examinations were performed incubating a feces sample for 24 hr at 37 C in 50 ml of selenite F broth. Each culture was plated in two petri plates containing 20 ml of Hektoen Enteric agar and two plates containing 20 ml of Brilliant Green agar. Ten suspect colonies

isolated from each agar were tested by the micro ID system (General Diagnostic) and minitek system (Becton Dickinson) to identify the different biotypes of *E. coli* by fermentation and enzymatic reaction patterns.

Antibiotic Sensitivity Tests. All *E. coli* isolates were assayed for drug resistance by the Kirby-Bauer method (Bauer *et al.*, 1966).

Antibiotic Resistance Transfer. The transfer of antibiotic resistance was carried out as per Datta (1965) method, utilizing as the receiving strain *E. coli* K 12 E 711 F-, sensitive to the four antibiotics under examination but resistant to high concentrations of nalidixic acid (100 μg/ml).

RESULTS

Antibiotic Treatment. The *E. coli* flora changes observed in chickens after oral administration of CTA, CAF, RN, and AMX at two different dosages and in the control groups are reported in Table 1. All *E. coli* biotypes isolated from different groups of chickens before treatment with lower dosages of antibiotics showed sensitivity to the antibiotic under examination. After the 1st, 2nd, and 3rd week of treatment, most *E. coli* isolates showed antibiotic resistance, but this resistance disappeared soon after the interruption of antibiotic administration.

After oral administration of the four antibiotics at higher dosages, the results are almost overlapping in the number of *E. coli* isolated biotypes and in the onset and disappearance of resistances during and after treatment. In all the control groups no significant changes were observed in the number of *E. coli* biotype isolates or in the sensitivity to the antibiotics throughout the period of the experiments.

Antibiotic Resistance Transfer. Data concerning the antibiotic resistance transfer in *E. coli* isolates are reported in Table 2. A high percentage of *E. coli* strains showed drug resistance to the antibiotic under examination. No significant difference in the detection of resistant strains was demonstrated following the two different dosage administrations. High percentage of resistant *E. coli* strains transferred their antibiotic resistance by conjugation and such transfer was observed mainly for CTA resistance. Transfer of RN resistance was not tested, because this resistance is not mediated by plasmids.

DISCUSSION

In a previous epidemiological study, we observed that a high percentage of *E. coli* (86.5%) isolated from avian feces were resistant to one or more antibiotics (Scioli *et al.*, 1980). Such development of multiple antibiotic resistant strains in domestic animals could be due to the use of antibiotics as feed additives

TABLE 1. *Number of E. coli biotypes isolated before, during, and after treatments with tetracycline (CTA), chloramphenicol (CAF), rifampicin (RN), and amoxicillin (AMX) administered at two different dosages and control groups*

Dosage	Before	After 7 days	After 14 days	After 21 days	3 Days after interruption	7 Days after interruption
CTA						
110 mg	5	4 (2)[1]	2 (2)	3 (2)	4	3
440 mg	4	4 (2)	2 (2)	2 (2)	3	2
Control	5	3	4	3	3	4
CAF						
110 mg	4	2 (2)	5 (5)	2 (2)	3	4
440 mg	3	4 (4)	3 (3)	3 (3)	2	3
Control	4	3	3	2	3	3
RN						
40 mg	7	7 (2)	7 (3)	6 (5)	6	6
80 mg	5	4 (2)	5 (3)	5 (4)	4	4
Control	7	6	6	6	7	6
AMX						
200 mg	4	2 (1)	1 (1)	2 (2)	2	2
400 mg	3	3 (2)	3 (2)	2 (2)	2	2
Control	3	3	2	3	3	2

[1] Number of resistant biotypes in parentheses.

TABLE 2. *Number and percentage of resistant and resistance-transferring E. coli strains isolated at the end of the 1st, 2nd and 3rd week of each treatment*

Antibiotic administration[1]	*E. coli* strains	Resistant strains	Transferring resistance strains
CTA (110 mg)	116	106 (91%)	51 (48%)
CTA (440 mg)	118	108 (91%)	59 (54%)
CAF (110 mg)	120	120 (100%)	32 (26%)
CAF (440 mg)	116	116 (100%)	24 (20%)
RN (40 mg)	110	64 (58%)	. . .
RN (80 mg)	112	76 (67%)	. . .
AMX (200 mg)	110	104 (94%)	33 (31%)
AMX (400 mg)	114	103 (90%)	31 (30%)
Total	916	797 (87%)	230 (35%)

[1] CTA, tetracycline; CAF, chloramphenicol; RN, rifampicin; and AMX, amoxicillin.

for growth promotion and prevention of disease.

The present study seems to confirm these data. In fact, after administration of the four antibiotics a high prevalence of resistant *E. coli* (87%) was detected from chicken feces. This suggests that the administration of antibiotics causes a selective pressure, which explains the detection of resistant bacteria. The interruption of antibiotic administration causes, as early as 3 days later, the disappearance of resistant strains and the establishment of the sensitive pre-existing flora. No significant difference was observed in detection of resistant *E. coli* after the use of low or high dosages of the antibiotics. Thus, the high percentage of antibiotic resistant strains of *E. coli* in domestic animals could be explained if antibiotics are administered, even in low dosage, without interruption.

Transfer of CTA resistance (48 to 54%) to receiving strains of *E. coli,* was frequent, whereas CAF and AMX resistance was transferred less frequently (20 to 26% and 30 to 31%, respectively). The finding of many antibiotic resistant strains of *E. coli* in chicken feces is in agreement with other reports (Gunaratne *et al.,* 1975; Levy *et al.,* 1976). And, if the passage of animal strains of *E. coli* to man occurs commonly (Smith, 1969; Levy *et al.,* 1976), the proportion of strains with transferable drug resistance is disturbingly high and must lend weight to efforts to control the use of antibiotics in animal feed.

Some authors have reported the presence of antibiotics, although in low concentrations, in domestic animal tissues (Michel-Briand, 1977). Thus, it might be advisable to inspect to observe the presence of antibiotic residues in chickens not officially allowed to receive feed that contains antibiotics.

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