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Possible Effect of Antibiotic-Supplemented Feed and Environment on the Occurrence of Multiple Antibiotic Resistant *Escherichia coli* in Chickens

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Abstract: The purpose of this study was to determine the occurrence of antibiotic resistant *Escherichia coli* isolated from chicks and chickens. This study was carried out on three flocks of birds fed commercial feeds supplemented with antibiotics from three commercial farms. The chicks and chickens in the fourth flock were reared in a chicken house, given feed without antibiotic supplementation. Cloacal swabs were taken from 50 birds per flock at 1, 21 and 42-day old. A total of 507 *E. coli* were isolated from these birds. The resistance of *E. coli* isolated form 1-day-old chicks to chloramphenicol (10 µg), cephalothin (30 µg), cephalaxin (30 µg), enrofloxacin (5 µg) and neomycin (30 µg) was 0-45% compared to the other four antibiotics, nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg) and trimethoprim (5 µg) which was 75-100%. The rates of resistance to antibiotics increased with the age of the chicks. Most of the isolates were resistant to at least 6 to 7 antibiotics. The highest rates of resistance to antibiotics were seen in 21 and 42 day old chickens. *Escherichia coli, Klebsiella* and *Pseudomonas* sp. isolated from feed samples were resistant to 4-9 antibiotics. The study suggests that the colonization antibiotic-resistant *E. coli* in the intestinal tracts of chicks and chickens were not necessarily due to the use of antibiotics in the feed as supplementation but may also be acquired from the immediate "contaminated" environment.

Key words: *Escherichia coli*, antibiotic resistance, chickens

INTRODUCTION

Antibiotics are used in veterinary medicine for treatment, as prophylaxis against production-threatening infectious diseases and in livestock feed at subtherapeutic doses to promote growth and increase feed efficiency. However, the use of antibiotics in animals is not totally safe. One of the main concerns is the development of antibiotic resistant bacteria. In fact, the use of antibiotics in food-producing animals is a contributory factor in the emergence of antibiotic-resistant bacteria in humans (JETACAR, 1999).

In poultry and pig production, rampant use of antibiotics has resulted in the development and maintenance of populations of antibiotic-resistant gram-negative enteric bacilli in the intestinal tracts of these animals (Mamber and Katz, 1985), making them reservoirs for resistant bacteria. Many of these bacteria owe their resistance to the extrachromosomal genetic elements, such as resistance (R)-plasmids, transposons and integrons that carry the resistance genes. It is usual for plasmids to carry a number of genes conferring resistance to several antibiotics and it is becoming increasingly common to find more than one resistant plasmid in bacteria (Smith and Lewin, 1993). The clinical significance of these phenomena is that the selective pressure for resistance caused by using antibiotics may result in multiple antibiotic resistance and these antibiotic resistant bacteria are known to be transmissible from animal to man.

The purpose of this study was to determine the antibiotic resistance of *E. coli* in chicks and chickens given commercial feeds supplemented with antibiotics (antibiotic-supplemented feeds) and in chicks and chickens given feed without antibiotics.

MATERIALS AND METHODS

Flocks and samples collections: Three commercial farms (5000-20,000 birds per farm), were selected in which chicks and chickens were given commercial feed known to contain antibiotics. Each farm used feed from different manufacturers. The label on the feed packaging did not specify the type nor the amount of antibiotic added as supplement. The manufacturers were not willing to provide such information. In each farm, 50 chicks or chickens (designated as Flock A, B, C) were sampled at random. The birds in Flock D were house separately and given feed without antibiotic supplementation.

Cloacal swabs were taken from each flock during the visits to the farms. Three sampling were done on each flock during each visit, that is when the birds were 1, 21 and 42 day old (slaughter age). In flocks A and B, cloacal swab samples from 1-old chicks were taken 12 h after hatching, before they were given feed or water. Flock C was sampled between 4-5 h and flock D, 18 h after hatching. Feed were also sampled for determination of antibiotic-resistant bacteria.

Isolation and identification: Each cloacal swab was directly streaked onto Chromocult Coliform agar (Merck) and the plates incubated at 37°C for 24 h. This agar is a selective medium that allows differentiation of *E. coli* from other coliforms and non-coliforms by the blue colour of the colonies. Other coliforms (namely species of *Citrobacter*, *Enterobacter* and *Klebsiella*) showed red coloured colonies, while non-coliforms colonies were colourless. *Escherichia coli* isolates were confirmed by Kovac's reagent (Merck), which caused the colonies to turn red.

Antibiotic susceptibility test: The antibiotic sensitivity test was performed by the agar disc diffusion method on Mueller Hinton agar (Oxoid). Bacterial cultures were prepared by inoculating the colonies into sterile distilled water to give an inoculum turbidity equivalent to a 0.5 McFarland turbidity standard and swabbed evenly onto agar plates and allowed to dry. The isolates were tested against nine antibiotics on disks, as follows: chloramphenicol (Ch) 10 µg, ciprofloxacin (Ci) 5 µg, cephalothin (Ce) 30 µg, cephalaxin (Cx) 30 µg, enrofloxacin (En) 5µg, neomycin (Ne) 30 µg, nalidixic acid (Na) 30 µg, 30 µg, streptomycin (St) 10 µg, tetracycline (Te) 30 µg and trimethoprim (Tr) 5 µg (Oxoid). The plates were incubated at 37°C for 24 h. Inhibition zone sizes were measured and interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (2002).

RESULTS

A total of 507 *E. coli* were isolated from 600 chicks and chickens, that is, 107 from 1 day old chicks, 200 each from 21 and 42 day old chickens. Flocks A, B, C and D produced 136, 128, 101 and 142 *E. coli* isolates respectively (Table 1). The occurrence of *E. coli* in 1 day old chicks varied according to time of hatching and sample collection. Thirty-six (72%), 28 (56%), 1 (2%) and 42 (82%) *E. coli* were isolated from one-day old chicks in flocks A, B, C and D, respectively. The number of *E. coli* isolates showing resistance to all nine antibiotics tested were 5 (4.7%) in one-day old chicks, 22 (11%) in 21 day old and 29 (14.5%) in 42-day old chickens or in 11.0% of total *E.coli* isolates while only 0.4% were resistant to one antibiotic. Of the 507 isolates, 1.0% were resistant to 2 antibiotics, 2.2% to 3 antibiotics, 2.8% to 4 antibiotics, 7.5% to 5 antibiotics, 10.7% to 6 antibiotics, 9.1% to 7 antibiotics and 7.1% to 8 antibiotics (Table 2). The resistance of *E. coli* isolates in 1 day old chicks to Ch, Ce, Ci, En and Ne were nil to moderate (0-45%). However, the resistance to Na, St, Te and Tr, was rather high at 75-100%.

The rates of resistance to antibiotics increased with the age of the chickens. In general, at one-day old, resistance to antibiotics were shown to 6-7 antibiotics and at 21 and 42 old, resistance were shown to 8-9 antibiotics. The highest rates of resistance to these antibiotics were in isolates from Flock D. In fact in this flock, particularly in the 21 and 42 day old chickens, almost all isolates were resistant to at least one antibiotic. One-day old chicks generally showed the least amount of antibiotic resistance.

Feed were also sampled to isolate enteric bacteria. *Escherichia coli*, *Klebsiella* and *Pseudomonas* sp. that were isolated from the feed were found resistant to between four to nine antibiotics.

DISCUSSION

The bacterial colonization of the intestinal tracts of chickens can occur as early as 3 h after hatching. The dynamics of such colonization depends on the microorganisms, their diversity and number increase with age of the birds (Barnes *et al.*, 1972; Barnes, 1977; Mead and Adams, 1975).

It was reported earlier that feed supplementation with antimicrobial agents appeared to have little influence on the occurrence and levels of antimicrobial-susceptible and antimicrobial-resistant gram-negative enteric bacilli (Mamber and Katz, 1985). This may not be true as this study showed that 1 day old chicks were colonized by antibiotic resistant *E. coli* even before exposure to feed and water. In another study, tetracycline-resistant *E. coli* was isolated in 1 day old chicks which were never given tetracycline in the feed and this resistant strains continued to be isolated for as long as 9 weeks of age (Kiser *et al.*, 1969). These observations suggested that environmental sources may be the main factors in the colonization of the chicks' intestinal tracts by the bacteria. Among the environmental sources of the bacteria include shell, feed, water, air, container for transportation and the workers themselves. Among the common bacteria that can be isolated from 1 day old chicks are antimicrobial-susceptible and -resistant *Klebseilla pneumoniae* and *Pseudomonas* sp. from the immediate environment and antimicrobial-susceptible and -resistant *Proteus mirabilis* from the feed (Mamber and Katz, 1985).

In our study, the *E. coli* isolated were resistant to at least one antibiotic. The majority of the isolates were resistant to at least six antibiotics. The resistant pattern seems to be age-dependent with the 21 and 42 day old chickens showing greater resistance than 1 day old chicks. The use of antibiotics in feed may have provided selective pressure resulting in a larger proportion of *E. coli*, *P.*

Table 1: Percentage of *Escherichia coli* isolated from chicks and chickens showing resistance to types of antibiotics

	Flock											
	1-day old				21-day old (n = 50)				42-day old (n = 50)			
Antibiotics (µg)	A (n = 36)	B (n = 28)	C (n = 1)	D (n = 42)	A	B	C	D	A	B	C	D
Cephalothin (5)	0	0	0	17*	42	50	50	50	50	50	50	50
Chloramphenicol (10)	11	11	0	43	98	66	68	54	96	78	78	86
Cephalaxin (30)	25	14	0	31	100	88	78	2	98	72	84	32
Enrofloxacin (5)	22	18	0	45	90	84	74	22	86	74	78	28
Neomycin (30)	17	14	0	45	90	100	82	44	58	78	92	68
Nalidixic acid (30)	80.5	96	0	93	100	100	94	80	100	100	98	100
Streptomycin (10)	83	75	0	76	100	98	90	34	98	8	94	58
Tetracycline (30)	92	96	0	86	100	100	84	84	100	96	98	96
Trimethoprim (5)	89	96	0	76	100	100	90	72	100	96	88	62

n = number of isolates from 50 chickens sampled per flock (Total: 507 isolates), Flocks A, B, andC were from 3 different farms; Flock D was housed separately and given feed without antibiotic supplement. *Refers to percentage of *E. coli* (isolated from each flock and age)

Table 2: Antibiotic resistance of *Escherichia coli* isolated from chicks and chickens based on number of antibiotics

	Flock											
No. of antibiotics *E.coli* resistant	1-day old				21-day old (n = 50)				42-day old (n = 50)			
	A (n=36)	B (n=28)	C (n=1)	D (n=42)	A	B	C	D	A	B	C	D
9	1 (3%)*	2 (7%)	0	2 (5%)	5 (10%)	8 (16 %)	7(14%)	2 (4%)	4 (8%)	8 (16%)	9 (18%)	8 (16%)
8	4 (11%)	2 (7%)	0	3 (7%)	0	1 (2 %)	5(10%)	2 (4%)	1 (2%)	5 (10%)	2 (4%)	11 (22%)
7	7 (19%)	5 (17%)	0	6 (14%)	0	4 (8 %)	3(6%)	6 (12%)	0	2 (4%)	1 (2%)	12 (24%)
6	9 (25%)	7 (25%)	0	11 (26%)	0	1 (2 %)	1(2%)	15 (30%)	0	2 (4%)	1 (2%)	7 (14%)
5	7 (19%)	9 (32%)	0	6 (14%)	0	0	4(8%)	4 (8%)	0	1 (2%)	1 (2%)	6 (12%)
4	2 (6%)	2 (7%)	0	0	0	0	0	9 (18%)	0	0	0	1 (2%)
3	0	1 (4%)	0	3 (7%)	0	0	0	5 (10%)	0	0	0	2 (4%)
2	0	0	0	1 (2%)	0	0	1(2%)	3 (6%)	0	0	0	0
1	0	0	0	0	0	0	0	2 (4%)	0	0	0	0

n = number of isolates from 50 chickens sampled per flock (Total: 507 isolates), Flocks A, B, and C were from 3 different farms; Flock D was housed separately and given feed without antibiotic supplement. Antibiotics: chloramphenicol, cephalothin, enrofloxacin, cephalaxin, neomycin, nalidixic, streptomycin, tetracycline, trimethoprim, *Refers to the number (percentage) of *E. coli* (isolated from each flock and age group) resistant to numbers of antibiotics

mirabilis, K. pneumoniae and *Pseudomonas* sp. in poultry, pigs and calves becoming resistant to one or more therapeutically useful antibiotics (JETACAR, 1999). One review suggested that bacteria can overcome "the cost of resistance" through compensatory mutations, which may explain why antibiotic resistance persists in the absence of drug selection pressure (Keyes *et al*., 2003). Many antimicrobial agents, particularly crude antimicrobial preparations have been used extensively as animal feed additives. In this form they act as carriers for the cognate resistance genes, providing opportunity for resistant bacteria to evolve and create an enormous gene pool for antimicrobial resistance in the environment (Lu *et al*., 2004).

Antibiotics are still continually and excessively used in both veterinary and human medicine. One major concern for this practice is the development of multi-antibiotic-resistant (MAR) *E. coli*. The subtherapeutic use of antibiotics in mass production of poultry meat, eggs and pork has promoted the emergence of the MAR *E. coli* in the faecal environment of these animals (Novick, 1981). The problem does not stop there. In poultry farms, wild birds and rodents consume antibiotic-containing feed or poultry droppings contaminated with MAR *E. coli*. Further down the line of the food chain, predatory animals, such as foxes, snakes and stray dogs also feed on rodents, escaped chickens and discarded chicken carcasses (Krumperman, 1983). These feeding behaviours cause the spread and maintenance of MAR *E. coli* in the environment. In addition raw chicken manure used as crop fertilizers further contaminate the environment with MAR *E. coli*.

Although antibiotic-supplemented feed may seem to be one of the causes in development of antibiotic-resistant bacteria, it should not be overlooked that resistant organisms and food borne pathogens could have been acquired from the contaminated environment due to drug use in a previous herd or flock of animals, contact with other animals during transportation to the slaughterhouses through cross-contamination during processing (Linton, 1986; Mamber and Katz, 1985). In newly hatched chicks, the common bacteria in the environments, whether antibiotic susceptible or resistant, colonize the intestines and become part of the intestinal normal microflora and proliferate whether or not the chicks are fed with antibiotic-supplemented feed. Upon colonization of *E. coli* in the intestines, the bacteria transfer antimicrobial resistant determinants to other bacteria, including potential pathogens in the intestines (Schroeder *et al*., 2004). The 1999 Report of the Joint

Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR), not only agreed that there are several evidence for the emergence of resistant bacteria in humans and animals following antibiotic use, but also for the spread of resistant animal bacteria to humans, the transfer of antibiotic-resistance genes from animal bacteria to human pathogens and resistant strains of animal bacteria causing human disease.

Conclusion: The judicious or prudent use of antibiotics in animals should be enforced to ensure that the risk of development of resistant commensals and zoonotic pathogens in animals and their spread in the environment is reduced. This in turn will lessen the risk to human health as the result of handling of animals and through consumption of contaminated food.

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