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Direct transmission of *Escherichia coli* from poultry to humans

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SUMMARY

Eight hundred and sixty-four *Escherichia coli* isolates from workers at the University of Ibadan Teaching and Research Poultry Farm, and 216 isolates from poultry attendants at a commercial poultry farm in the city were found to be resistant to streptomycin, sulphafurazole and tetracycline. In contrast, all 576 and 288 *E. coli* isolates from village fowls and from villagers respectively were sensitive to these drugs. Isolates from birds in a modern university poultry unit (3744) exhibited the same resistance patterns as those isolated from workers who were in direct contact with the birds. No nalidixic acid-resistant *E. coli* was isolated from farm workers prior to their assignment to the experimental pen. Following experimental oral infection of birds with *E. coli* K12 J5 NA^+ Lac^-, the organism was recovered from the workers who manned the experimental pen. Neither before nor after the experimental infection was any nalidixic acid-resistant *E. coli* isolated from workers who manned the pen from which birds used in the experiment were selected. Similarly, no drug resistant organisms were isolated from workers outside the poultry unit of the university or commercial farm. The MIC of the drugs against the avian and human *E. coli* isolates at the university and commercial poultry farms were similar.

INTRODUCTION

In animal husbandry, antibiotics are used as prophylactics and as growth promoters, particularly where animals are reared under intensive husbandry practice. Such non-therapeutic uses have been of major concern to those engaged in the treatment of infectious diseases, especially as a correlation has been found between the widespread use of antimicrobial drugs and the emergence of drug resistance (1–18). Thus, resistance patterns of animal *E. coli* isolates have been found to be similar to those found among the isolates from humans who were closely associated with the animals (7, 14, 17–20). In the tropical developing countries, where antibiotics are very readily available without a prescription, and where the environmental sanitation is poor, cross infection, which was reported in Britain by Linton (12) and in the United States by Levy and colleagues (21) and Holmberg and co-workers (18), could conceivably occur on a large scale. For

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instance, in Nigeria, resistant *E. coli* have been isolated in large numbers from antibiotic-fed poultry (14). This investigation was undertaken to ascertain whether avian *E. coli* may be transmitted to humans who are in direct contact with the birds.

MATERIALS AND METHODS

Stool/cloacal swab samples were collected from the following:

(*a*) Four workers at a special poultry pen (the experimental pen) University of Ibadan Teaching and Research Agricultural Farm. These workers manned the experimental pen, and were in direct contact with the experimentally inoculated birds.

(*b*) Thirty-six birds kept in the experimental pen on the university farm.

(*c*) Eight workers elsewhere within the poultry unit of the university farm. These workers had no contact with the birds in the experimental pen.

(*d*) Five workers on the university farm, at various sites outside the poultry unit.

(*e*) Three attendants at a specially designated pen (experimental pen) at a commercial farm, Old Ife Road, Ibadan.

(*f*) Sixteen birds in the specially designated experimental pen at the commercial farm.

(*g*) Two workers at different sites outside the poultry unit of the commercial farm.

(*h*) Four villagers in each of three villages, Aba Alaja, Aba Awusa and Aba Fakore, on the outskirts of Ibadan city.

(*i*) Twenty-four fowls in the three villages.

On both the university and commercial poultry farms, birds were kept in pens with deep floor litter, which consisted of sun-dried grass and sawdust from carpentry workshops. In pens where only broilers were kept, no cages were provided, but where there were egg layers, cages were installed. The cages, built like book shelves in two or three layers, and with easy access for the laying birds, were attached to wooden frames at one end of the pen such that the lower cages stood 40–50 cm above the floor. In the villages however, no accommodation was provided for the fowls, which found lodging sites in dilapidated or abandoned buildings, beside outer walls of their owners' houses or in shrubs and bushes nearby.

The poultry husbandry practice on both the university and commercial farms was similar, emulating the intensive rearing system of Europe and the United States, often supplementing their feeds with antimicrobials, tetracycline and sulphadimidine, at levels of 100 ppm. In contrast, free-range village fowls received no antibiotics; in fact they were rarely fed by their owners, and they fended for themselves, scavenging in shrubs and bushes around the villages.

Isolation and confirmation of Escherichia coli

Cloacal swabs and stool samples were inoculated onto McConkey agar, and after overnight incubation at 37 °C, colonies morphologically resembling *E. coli* were carefully picked and sub-cultured onto nutrient agar to check for purity (22). A single colony from each pure culture was then sub-cultured into peptone

water, incubated for 4 h at 37 °C, and identity confirmed by biochemical tests (23).

Bacteriological methods

A new pen with fresh deep litter was prepared and left unused for 2 weeks after which it was bacteriologically examined twice weekly for 2 months (24). Samples of floor litter were collected from several locations, and at different times of the day, in wet as well as dry portions. Sterile spoons were used to collect about 20 g portions each of floor litter which were placed in sterile conical flasks and suspended in peptone water. The flasks were allowed to stand for several hours after which the supernatant was cultured for *E. coli* as previously described.

Thirty-six birds, from which *E. coli* resistant to streptomycin, sulphafurazole and tetracycline had been isolated, were transferred into the experimental pen. Their bacteriological status was then determined by culturing cloacal swabs before an experimental oral inoculation was carried out. Sixteen birds at the commercial farm were treated in a similar manner. Stools of four workers, who were delegated to man the new pen were bacteriologically examined twice weekly for one month before they were assigned to the new experimental pen. At the commercial farm, three attendants were simultaneously examined bacteriologically. At the village level, the free-range fowls and the villagers were routinely examined bacteriologically as their birds were not exposed to antibiotics. As controls on both the university and commercial farms, birds that harboured resistant *E. coli*, but which were not inoculated with *E. coli* K12, as well as all the workers who had no contact with the experimentally inoculated birds were examined concurrently.

E. coli was isolated weekly for 8 weeks from workers and from birds before experimental inoculation of the birds with *E. coli* K12 J5 NA^+ Lac^- on both the university and commercial farms. Stool samples were collected from the workers on alternate days while cloacal swab samples were taken from the birds daily. At the village, stool samples were collected from the villagers once a week, and cloacal swabs taken from fowls once weekly for 12 weeks.

Experimental oral inoculation with E. coli *K12 J5 NA^+ Lac^-*

A colony of *E. coli* K12 J5 NA^+Lac^-, a laboratory strain that was nalidixic acid resistant, lactose negative, and sensitive to streptomycin, sulphafurazole and tetracycline, was inoculated into 10 ml nutrient broth and incubated at 37 °C for 24 h; a suspension containing about 10^5 organisms per ml was prepared and 1 ml given to each bird directly into the oesophagus with the aid of a dropping pipette. Cloacal swabs from these birds were examined bacteriologically 24 h after the initial inoculation, and twice daily thereafter the following 10 days. These steps were repeated at the commercial farm with 16 birds.

The Escherichia coli *isolations studied*

In all, 6192 *E. coli* isolations were made as follows:

(*a*) Workers at the university experimental pen – 128 isolations before, and 160 after experimental oral inoculation of the birds.

(*b*) Birds in the university experimental pen – 1152 isolations before, and 1140 after experimental oral inoculation.

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(*c*) Workers within the poultry unit, but employed elsewhere on the university farm – 256 isolations before, and 320 after the experimental oral inoculation of the birds.

(*d*) Workers outside the poultry unit, but engaged somewhere else on the university farm – 160 isolations before, and 200 after experimental oral inoculation of the birds.

(*e*) Attendants at the experimental pen of the commercial farm – 96 isolations before, and 120 after the experimental oral inoculation of the birds.

(*f*) Birds in the experimental pen of the commercial farm – 512 isolations before, and 640 after experimental inoculation of birds.

(*g*) Workers outside the poultry unit of the commercial farm – 64 isolations before, and 80 after experimental oral incoulation of the birds.

(*h*) Villagers in the three villages – 288 isolations.

(*i*) Free-range fowls in the three villages – 576 isolations.

Sensitivity tests

A single colony of each pure culture of the isolated *E. coli* was inoculated into nutrient broth and incubated at 37 °C overnight. Using a standard loop, approximately 0·001 ml of the suspension was transfered to 1 ml of sterile $\frac{1}{4}$ strength Ringer's solution, and the resulting suspension which contained approximately 10^6 organisms per ml was used to produce a lawn inoculum on 90 mm diameter Petri dishes containing 25 ml of Oxoid Sensitivity Test Agar (CM 261). This inoculum size produced dense, but just not completely confluent growth (14, 25, 26). The agar surface was allowed to dry, an Oxoid Multodisk was then placed in position, and the plates incubated at 37 °C overnight. Resulting zones of inhibition were measured the following morning. The Oxoid Multodisk contained the following agents (μg): ampicillin, 25; nitrofurantoin, 200; chloramphenicol, 50; streptomycin, 25; colistin, 10; sulphafurazole, 500; nalidixic acid, 30; tetracycline, 50.

Minimal inhibitory concentrations

The minimal inhibitory concentrations (MIC) of streptomycin, sulphonamide and tetracycline against the avian and human *E. coli* isolates at the university and commercial farms were estimated using the agar dilution method (26), incorporating the drugs into agar. The final concentrations of antimicrobials incorporated in the sensitivity agar were: streptomycin, 32, 16, 8, 4, 2 and 1 μg/ml; sulphonamide, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0·5 μg/ml; tetracycline, 8, 4, 2, 1 and 0·5 μg/ml.

For each batch of tests, control plates of Sensitivity Test Agar without antibiotic were also prepared.

Estimation of MIC

Each *E. coli* isolate was inoculated into nutrient broth, incubated at 37 °C overnight, and then diluted in $\frac{1}{4}$ strength Ringer's solution to give an approximate density of 10^6 organisms per ml. The control *E. coli* NCTC 10148 was included in each batch of tests. Using a standard 0·01 ml platinum loop, each isolate was inoculated as a point inoculum onto each antibiotic-containing plate, and onto the

Table 1. *Sensitivity tests of* Escherichia coli *isolates from various avian and human sources*

	Percentage sensitive								
	A	B	C	D	E	F	G	H	I*
Chloramphenicol (50 μg)	100	100	100	100	100	100	100	100	100
Colistin (10 μg)	100	100	100	100	100	100	100	100	100
Nitrofurantoin (200 μg)	100	100	100	100	100	100	100	100	100
Sulphafurazole (500 μg)	0	0	97	98	0	0	100	97	100
Nalidixic acid (30 μg)	100	100	100	100	100	100	100	100	100
Ampicillin (25 μg)	96	100	98	98	100	100	100	100	100
Streptomycin (25 μg)	0	0	16	12	0	0	17	99	98
Tetracycline (50 μg)	0	0	72	73	0	0	74	97	96
No. of *E. coli* isolates tested	128	1152	256	160	96	512	64	288	576

* See text for details.

Table 2. *Drug resistance among avian and human* E. coli *isolates*

Source of *E. coli** no. of isolates)	Percentage resistant		
	Streptomycin	Sulphonamide	Tetracycline
A (288)	100	99	100
B (2592)	100	96	100
C (576)	85	97	28
D (360)	88	94	27
E (216)	99	97	26
F (1152)	100	96	100
G (144)	83	95	26
H (288)	0·3	0	0·7
I (576)	0·2	0	0

* See text for key.

control plate containing no antibiotic. All plates were incubated at 37 °C overnight, after which they were examined for growth. The MIC was taken as the lowest concentration of antibiotic which gave complete inhibition of bacterial growth.

RESULTS

Sensitivity tests

Table 1 shows the level of sensitivity and resistance exhibited by *E. coli* isolates from avian and human sources. There is a clear difference between the sensitivity of isolates from battery poultry and free-range village poultry. Columns B, F and I show that while all isolates from free-range village poultry were sensitive to all microbials tested (98% in the case of streptomycin, and 96% in the case of tetracycline), all isolates from modern university and commercial battery poultry were resistant to streptomycin, sulphafurazole and tetracycline. Isolates from workers in close association with the birds showed similar sensitivity/resistant patterns (see Table 1, columns A and E). In sharp contrast, practically all isolates

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Table 3. *MICs (μg/ml) of various drugs against avian and human* E. coli *isolates**

	University farm		Commercial farm			
Group	B	A	F		E	
	E. coli isolates		*E. coli* isolates			
n ...	1152	128	512		96	
	Avian	Human	Avian		Human	
Streptomycin						
MIC	32	32	32		32	
% of isolates	100	100	100		100	
Sulphafurazole						
MIC	512	512	256	512	256	512
% of isolates	100	100	0·2	99·8	0·3	99·7
Tetracycline						
MIC	8	8	4	8	4	8
% of isolates	100	100	0·8	99·2	0·5	99·5

For comparison, MICs of streptomycin, sulphonamide and tetracycline against control *E. coli* NCTC 10148 are 4, 4 and 2 μg/ml respectively.

* See text for details.

from villagers were sensitive to all agents tested. Table 2 shows the level of resistance against the three drugs, streptomycin, sulphafurazole and tetracycline, often used as feed additives with a view to utilizing them as prophylactics and growth promoters.

Minimal inhibitory concentrations

Table 3 shows that the MICs of streptomycin and tetracycline for the avian and human *E. coli* isolates were four times higher than those for *E. coli* NCTC 10148. As for sulphonamide, the MICs required to inhibit the avian and human *E. coli* isolates from battery farms were 128 times higher than those for control *E. coli* NCTC 10148.

Litter

No drug resistant *E. coli* was isolated from the litter on either the university or commercial poultry farm.

Experimental oral inoculation and recovery of E. coli *K12*

E. coli K12 J5 NA^+Lac^- was recovered from 28 of the 36 inoculated university pen birds 24 h post inoculation. From the third day post inoculation, and the following 10 days, the organism was recoverd from 16–30 of the birds (Table 4). Cloacal swab samples from all the birds were positive on the fourth day post inoculation. The *E. coli* isolated from the four workers prior to their assignment to the special experimental pen on the university farm, were resistant to streptomycin, sulphafurazole and tetracycline, but sensitive to chloramphenicol, colistin, furazolidone, nalidixic acid and ampicillin. However, one week after their assignment to the experimental pen, lactose negative *E. coli*, resistant to nalidixic

Table 4. *Recovery of* E. coli *K12 J5 NA*$^{+}$ *Lac*$^{-}$ *from humans and avians post-experimental inoculation of birds*

	Days post-experimental inoculation No. positive/no. of birds or workers examined									
	1	2	3	4	5	6	7	8	9	10
Three commercial farm attendants	—	3 (3)	3 (3)	3 (3)						
	—	—	—	3 (3)						
Thirty-six university farm birds	28 (36)	30 (36)	26 (36)	36 (36)	25 (36)	24 (36)	24 (36)	22 (36)	28 (36)	20 (36)
	28 (36)	25 (36)	24 (36)	30 (36)	27 (36)	26 (36)	21 (36)	25 (36)	22 (36)	16 (36)
Sixteen commercial farm birds	—	12 (16)	13 (16)	14 (16)	16 (16)	14 (16)				
	10 (16)	12 (16)	14 (16)	14 (16)	13 (16)	12 (16)				
Four university farm workers	—	—	—	2 (4)	2 (4)	2 (4)	2 (4)	3 (4)		
	—	—	—	2 (4)	2 (4)	3 (4)	2 (4)	3 (4)		

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acid, but sensitive to other drugs was isolated from three of the four, and sometimes from all four workers during the next 5 days. No nalidixic acid-resistant *E. coli* was isolated from the eight workers who manned the old pen housing birds from which the experimentally infected ones were selected.

Like their counterparts on the university farm, all three attendants on the commercial farm were free from nalidixic acid-resistant *E. coli* prior to their assignment to the specially designated experimental pen. Forty-eight hours after oral inoculation of the birds, nalidixic acid-resistant, lactose negative *E. coli* was recovered from 10 of the 16 birds, while stool samples from all three attendants yielded this organism over a period of 4 days. The organism was recovered 40 h post inoculation, and thereafter twice daily for 5 days from between 12 and all 16 birds (Table 4). No drug resistant *E. coli* was isolated from any of the eight other workers outside the poultry unit of the commercial farm.

No stool sample from any of the 12 villagers, or cloacal swab samples from any of their 24 fowls in the three villages yielded any drug-resistant *E. coli*.

DISCUSSION

Escherichia coli isolates from stools of poultry farm workers and from cloacal swabs from the birds of modern battery poultry showed similar resistance patterns, indicating that these workers probably acquired the drug resistant organisms from the birds. Examiniation of the workers prior to their first posting to the poultry pens showed that they did not harbour drug-resistant *E. coli*. Resistance patterns of the avian *E. coli* isolates were similar to those of isolates from humans who were closely associated with the modern battery poultry. This is in agreement with the findings of Wells & James (19), Fein and colleagues (20) and Marsik and colleagues (7). The results reported here indicate a direct avian to human transmission of *E. coli* K12 J5 $NA^{+}Lac^{-}$ which was experimentally orally inoculated into the birds and subsequently recovered from the workers who manned the experimental pen. Neither the workers nor the birds had previously harboured this organism.

Administration of antibiotics to animals over long periods for prophylaxis favours the persistence of resistant strains long after the selection pressure has been removed (27). The effect of such prolonged and continuous exposure has helped to stabilize resistant organisms which then appear as an integral part of the mormal flora of the gastro-intestinal tract. It is known that a large reservoir of antibiotic-resistant *E. coli* exists in poultry with the organisms being regularly excreted in the birds' faeces (14, 28) and these resistant *E. coli* can reach man through poultry products. The presence of these resistant organisms, and the possibility of their resistance being transferred to food poisoning organisms like the salmonellae are a potential hazard to human health.

Antibiotics have been given to animals extensively either as growth promoters or as prophylactics, and although it is, in principle, prohibited to use these drugs except under strict veterinary supervision, they are nevertheless still widely used. Swann's recommendations (29) were not given a chance to prove their worth in Nigeria for instance, as they were never applied. This non-therapeutic use must account partly for the large number of resistant *E. coli* present in the gastro-

intestinal tract of poultry (5, 8, 12, 14, 27, 30). Where the resistance factor contains genes for resistance to more than one antibiotic, the use of a single agent will select for multiple resistance (31) and this may complicate the therapy of veterinary as well as human infections. The commonest drugs used as feed additives in Nigeria are the broad spectrum tetracyclines; this group of antibiotics constitutes one of the most potent agents for provoking the emergence and selection of resistance plasmids (13).

Whether avian *E. coli* and other Enterobacteriaceae establish infections in humans will require experimental infections with a virulent bacterial strain, a procedure that has many complications, not least ethical, and must be approached with the greatest caution.

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