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Public Health Aspects of Bacterial Drug Resistance in Modern Battery and Town/Village Poultry in the Tropics

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Ojeniyi, A: Public health aspects of bacterial drug resistance in modern battery and town/village poultry in the tropics. Acta vet. scand. 1989, 30, 127–132. – A correlation of antibiotic use and drug resistance was found among *Escherichia coli* strains isolated from battery poultry in the University of Ibadan Teaching and Research Farm. All the *E coli* strains from battery birds were resistant to Tetracycline, Streptomycin and Sulphonamide (Sulphafurazole) by the disc sensitivity test. In contrast, all the strains isolated from free range town and village poultry were sensitive to the range of drugs tested, while the Minimal Inhibitory Concentrations of the drugs against these free range isolates were the same as those for the control *E. coli* strain N.C.T.C. 10418. Ninety-eight to 100 % of the strains from both battery poultry and from town/village birds were sensitive to Colistin, Chloramphenicol, Nitrofurantoin and Nalidixic Acid. Since antibiotic-resistant *Escherichia coli* from animal sources are known to be indistinguishable from those found in man, the indiscriminate use of antibiotics is potentially capable of giving rise to a higher incidence of intractable infection with resistant bacteria. These drug-resistant organisms may be transmitted from animal to man, and complicate the therapy of human diseases.

antibacterials; resistant *E coli*

Introduction

The widespread use of antibiotics, whether for therapy, prophylaxis or growth promotion, may lead to the emergence of antibiotic resistant bacterial populations in animals, including poultry (*Siegel et al* 1974, *Smith and Tucker* 1975, *Linton* 1977a & b, *Smith* 1978, *Ojeniyi* 1980, *Holmberg et al.* 1984a). The more indiscriminately antibiotics are used, the higher the incidence of intractable infection with resistant bacteria may become. Since resistant organisms from animals can also colonise and infect human beings, the use or abuse of antibiotics in animals may complicate the therapy of human disease, quite apart from the fact that elimination of sensitive bacterial strains and their replacement by antibiotic resistant organisms may lead to untreatable infections in the animals themselves. Tetracycline, one of the broad spectrum antibiotics, is widely and possibly uncritically used in poultry groups kept under the battery system in Ibadan, Nigeria and many other tropical developing countries.

Attempts have been made to evaluate the relationship between *Escherichia coli* strains of animal origin and human disease, and to assess the risk that they pose to man. Antibiotic resistant *E. coli* from animal sources have been found to be indistinguishable from those found in man, and it has also

been found that the use of antibiotics in animals, for whatever purpose, contributes to the number of resistant *Escherichia coli* transmitted from animals to man (*Linton* 1977a & b, *Harbour et al.* 1978, *W.H.O.* 1978). Antimicrobial resistant salmonella organism of animal origin has been shown to cause very serious clinical infections in human beings (*Holmberg et al.* 1984b).

In view of these findings, and because of the widespread local practice of incorporating antibiotics in poultry feeds, it was considered desirable to investigate the effect of antibiotic usage on *Escherichia coli* isolated from poultry in Ibadan and its environs.

Materials and methods

Cloacal swabs were collected from: –

(a) 500 birds at the University of Ibadan Teaching and Research Poultry Farm. These birds received antibiotics which were incorporated in their feeds.
(b) 100 birds caught in 3 villages – Aba Alaja, Aba Awusa and Aba Fakore – on the outskirts of Ibadan city. These birds are kept under free range conditions and were not exposed to antibiotics.
(c) 200 chickens collected in Igbo-Ora and Idere towns in Ibarapa Division of Oyo State, Nigeria. These chickens are occasionally fed by their owners, otherwise they fend for themselves by scavenging around the houses in the townships.

Bacteriological methods

Isolation of *Escherichia coli.* Swabs were inoculated onto MacConkey agar, and after overnight incubation at 37°C, isolated lactose fermenting colonies morphologically resembling *Escherichia coli* were subcultured to nutrient agar to check for purity. A single colony from each pure culture was then subcultured into peptone water, incubated for 4 h at 37°C, and then used for biochemical tests (*Cowan* 1975) for identification.

Disc Sensitivity Tests. Pure cultures of *Escherichia coli* were inoculated into nutrient broth and incubated at 37°C overnight, after which the cultures were transferred into 1 ml of sterile 1/4 strength Ringer's solution, and the resulting suspensions which contained approximately 10^6 organisms per ml were 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 (*W.H.O.* 1961, *Garrod et al.* 1973).

The agar surface was allowed to dry, an Oxoid Multodisc UI 40569-236 was then placed in position, and the plates incubated at 37°C overnight. Resulting zones of inhibition were measured the following morning using calipers and a ruler in the absence of a planimeter. The Oxoid Multodisc contained the following chemotherapeutic agents: –

Ampicillin	25 µg	Chloramphenicol	50 µg
Colistin	10 µg	Nalidixic Acid	30 µg
Nitrofurantoin	200 µg	Streptomycin	25 µg
Sulphafurazole	500 µg	Tetracycline	50 µg

Minimal Inhibitory Concentrations

The Minimal Inhibitory Concentrations of the various drugs were estimated, using the Agar Dilution Method (*Garrod et al.* 1973).

Preparation of plates. Ampoules of antibiotics for clinical use were employed as starting materials as follows: –

(i) Ampicillin 250 g ampoule (Beecham B.P.)
(ii) Streptomycin 1 g ampoule (Glaxo B.P.)
(iii) Sulphadiazine 1 g ampoule (May & Baker B.P.)
(iv) Tetracycline 250 g ampoule (Lederle B.P.)

For estimating Minimal Inhibitory Concentration of Chloramphenicol, the B.P. pure

powder (Parke Davis) was weighed out and dissolved in alcohol to a known concentration which was then further diluted in sterile water as required.

To prepare the final plates, 99 ml volumes of Oxoid Sensitivity Test Agar were melted and cooled to 45°C. One ml of the appropriate antibiotic dilution was added, and the antibiotic-containing agar poured into each of the petri dishes.

The final antibiotic concentrations incorporated in the Sensitivity Agar were: –

Ampicillin: 32, 16, 8 and 4 μg/ml.

Chloramphenicol: 32, 16, 8, 4, 2 and 1 μg/ml.

Streptomycin: 32, 16, 8, 4, 2 and 1 μg/ml.

Sulphadiazine: 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 Agar without antibiotic were also prepared.

Estimation of Minimal Inhibitory Concentrations. Each *E. coli* strain was inoculated into nutrient broth, incubated at 37°C overnight, and then diluted in 1/4 strength Ringer's solution to give an approximate density of 10^6 organisms. The control *E. coli* N.C.T.C. 10418 was included in each batch of tests.

Using a standard 0.01 ml platinum loop, each strain was inoculated as a point inoculum onto each antibiotic-containing plate, and onto the control plate containing no chemotherapeutic agent. The inoculum size used contained approximately 10^4 organisms.

All plates were incubated at 37°C overnight, after which they were examined for growth. The Minimal Inhibitory Concentration was taken as the lowest concentration of chemotherapeutic agent which gave complete inhibition of bacterial growth.

Results

Agents for which Minimal Inhibitory Concentrations were not estimated

Colistin. All strains of *E. coli* isolated from village poultry were inhibited by the 10 μg Colistin disc, with zone diameters ranging between 11 and 16 mm. There were 2 % of the *E. coli* strains isolated from poultry at the University of Ibadan Teaching and Research Farm which showed no inhibition zones, while the remaining 98 % produced zone diameters ranging from 12 to 16 mm. Ten replicate tests with the control *E. coli* N.C.T.C. 10418 gave zone sizes ranging from 14 to 16 mm. All, except the few uninhibited strains from the University poultry, were therefore considered sensitive to this agent.

Nitrofurantoin. Findings were similar to those for Colistin, with all, except 2 % of the strains from the University poultry giving inhibition zones ranging in size from 17 to 28 mm in diameter. Ten replicate tests using the control *E coli* N.C.T.C. 10418 gave zone sizes ranging from 20 to 23 mm in diameter. All the strains, except the uninhibited few, which were not the same as the Colistin-resistant strains, were therefore considered to be sensitive to Nitrofurantoin.

Nalidixic Acid. All strains, without exception, gave zones of inhibition ranging between 18 and 25 mm in diameter. Ten replicate tests using the control *E. coli* N.C.T.C. 10418 gave zone sizes of inhibition ranging between 23 and 26 mm in diameter. All strains therefore, be these from University poultry, town poultry or village poultry, were considered to be sensitive to Nalidixic Acid.

Table 1. Minimal Inhibitory Concentrations of various drugs against 500 *Escherichia* coli strains isolated from University battery poultry, 200 strains from town and 100 strains from village poultry.

	University battery poultry				Town poultry			Village poultry		
Ampicillin										
M.I.C. in µg per ml	4	8	32		4	8	32	4	8	32
% of strains with M.I.C.	65	22	13		84	16	0	88	12	0
Chloramphenicol										
M.I.C. in µg per ml	4	8	16	32	4	8	32	4	8	32
% of strains with M.I.C.	0	85	11	4	60	40	0	50	50	0
Streptomycin										
M.I.C. in µg per ml	32 or above				4			4		
% of strains with M.I.C.	100				100			100		
Sulphonamide										
M.I.C. in µg per ml	512 or above				4			4		
% of strains with M.I.C.	100				100			100		
Tetracycline										
M.I.C. in µg per ml	8 or above				2		4	2		4
% of strains with M.I.C.	100				96		4	94		6

For Control *Escherichia coli* N.C.T.C. 10418, M.I.C. of Ampicillin = 8, Chloramphenicol = 8, Streptomycin = 4, Sulphonamide = 4, Tetracycline = 2 µg/ml.

Agents for which Minimal Inhibitory Concentrations were estimated

The results of the Minimal Inhibitory Concentrations' estimation for Ampicillin, Chloramphenicol, Streptomycin, Sulphonamide and Tetracycline are shown in Table 1. It will be seen that all the strains from the University poultry were resistant to the highest concentrations of Tetracycline, Streptomycin and Sulphonamide tested, while all the strains from village poultry had the same M.I.C's as the control *E.coli* N.C.T.C. 10418 in the case of Streptomycin, Sulphonamide and Tetracycline.

All the village poultry strains had an M.I.C. of Ampicillin that was equal to, or less than that of the control *E. coli* while 22 % of the University strains were resistant to 32 µg/ml, i.e. 4-folds of the M.I.C. for the control *E. coli.*

In the case of Chloramphenicol, all the village strains had M.I.C. values of 8 µg/ml or less. The M.I.C. of Ampicillin for the control *E. coli* was 8 µg/ml. Among the strains from University poultry, there were 11 % with an M.I.C. of 16 µg/ml which may be considered similar to that of the control *E. coli* strain from which it differs by only a single two-fold dilution, while 4 strains had M.I.C's of 32 µg/ml or greater.

All the strains from University poultry had M.I.C.'s of Tetracycline that were 8 times or more that of the control *E. coli* strain, while 98 % of village strains had the same M.I.C. as that of the control *E. coli* N.C.T.C. 10418 strain.

Discussion

The widespread use of antibiotics, especially tetracycline, in battery poultry groups in a tropical country, like Nigeria, encourages

the proliferation of antibiotic-resistant bacteria.

Administration of antibiotics to animals over long periods for prophylaxis favours the persistence of resistant strains long after the selection pressure has been removed (*Harbour et al.* 1978). The effect of such prolonged and continuous exposure helps to stabilise resistant organisms which then appear as integral part of the gastro-intestinal tract flora. Public Health authorities naturally feel a concern for the potential hazard to human health posed by the drug-resistant organisms in poultry since these oranisms are regularly excreted in large numbers in the birds' faeces (*Howe et al* 1976) and can reach man through poultry products (*Burton et al.* 1974, *Harbour et al.* 1978). This is even more significant in the tropical developing countries where drugs are very easily available without prescription and are rather indiscriminately and uncritically used. Stricter veterinary and pharmacy control of drugs is advocated.

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Sammendrag

Konsekvensen for det offentliges sundhed af lægemiddelresistens hos bakterier blandt burhøns og løsgående landsbyhøns i troperne

Blandt moderne fjerkræhold i Nigeria forekom der *Escherichia coli*, der var resistent overfor streptomycin, tetracyclin og sulfonamider (sulfafurazol) i modsætning til *E coli* isolater hidrørende fra frigående landsbyhøns, der alle var følsomme for samme antimikrobielle midler. Mellem 98 og 100 % af isolaterne, fra såvel moderne fjerkræhold som fra landsbyhøns var følsomme overfor colistin, kloramfenikol, nalidixinsyre og nitrofurantoin. De minimale hæmningskoncentrationer (MIC) af diverse antibiotika og kemoterapeutika overfor landsbyhøns' *E coli* var de samme som for standard *E. coli* N.C.T.C. 10418. For moderne fjerkræholds *E coli* derimod havde 13 % af dem MIC af Ampicillin på 32 µg/ml (4 × MIC for standard *E. coli*), for kloramfenikol havde 11 % MIC på 16 µg/ml og 4 % havde 32 µg/ml (2 × henholdsvis 4 × MIC for standard *E coli*), for streptomycin var end ikke 32 µg/ml (8 × MIC for standard *E coli*) nok til at hæmme organismerne. Ligeledes var MIC af sulfonamid høj, 512 µg/ml (128 × MIC for standard *E coli*) og for tetracyklins vedkommende var MIC på 8 µg/ml (4 × MIC for standard *E coli*).

Da antibiotikaresistente animale *E coli* vanskeligt kan skelnes fra de, der forekommer hos mennesker, kan ukritisk brug af antimikrobielle midler hos husdyrene medføre en højere incidens af langvarige og svært behandlelige infektionssygdomme såvel hos dyr som hos mennesker.

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