Online Journal of Health and Allied Sciences

Published Quarterly
Mangalore, South India
ISSN 0972-5997
Volume 4, Issue 3; Jul-Sep 2005

Original Article

Anti Microbial Resistance Profile of *E. coli* isolates From Tropical Free Range Chickens

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Citation
Okoli CI, Chah KF, Ozoh PTE, Udedibie ABI. Anti Microbial Resistance Profile of *E. coli* isolates from Tropical Free Range Chickens. *Online J Health Allied Scs.*2005;3:3

URL
http://www.ojhas.org/issue15/2005-3-3.htm

Open Access Archives
http://cogprints.ecs.soton.ac.uk/view/subjects/OJHAS.html
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Abstract:
Normal intestinal flora of humans and animals constitute enormous reservoir of resistance genes for potentially pathogenic bacteria and may serve as major indictors of selection pressure exerted by anti-microbial use in a given population. A study was conducted in September 2003 at 3 purposively selected peri-urban sites spread across 3 senatorial zones of Imo state, Nigeria to determines the anti-microbial resistance profile of commensal E. coli isolated from free range chickens. The isolates were screened for anti-microbial resistance profile against 10 antibiotics using the disc diffusion method. E. coli strains from local fowls, recorded 100 and 78.9% resistances against ampicillin and cotrimoxazole respectively, while resistance rates against ciprofloxacin, gentamycin and norfloxacin were 0.0, 5.3 and 5.3% respectively. Isolates from free-range cockerels recorded 100% resistance against norfloxacin, cotrimoxazole and ampicillin and 83.3% against nitrofurantoin. Similarly, isolates from old layers, recorded 100% resistance against nitrofurantoin, cotrimoxazole, tetracycline, chloramphenicol and ampicillin and 80% against nalidixic acid. Similar resistance trends were observed in E. coli isolates from free-range turkeys and broiler roosters against the inexpensive broad-spectrum first line antibiotics (ampicillin, nalidixic acid, cotrimoxazole, nitrofurantoin and chloramphenicol), although values varied slightly across poultry types. Resistances against gentamycin were consistently low in isolates from the different types of poultry. Twenty-nine resistance patterns were observed in the E. coli isolates with predominant patterns being distributed widely across poultry types indicating a striking diversity of resistance patterns in the areas.
Key Words: *E. coli,* Anti-microbial resistance, Local fowl, Turkey, Roosters, Cockerels, Nigeria

Introduction

The level of anti-microbial resistance in bacteria belonging to the normal intestinal flora of humans and animals may increase due to exposure to antibiotics.(1) These bacteria which constitute an enormous reservoir of resistance genes for potentially pathogenic bacteria may serve as major indictors for selection pressure exerted by anti-microbial use in a given animal or human population.(1-3) Investigation of prevalence of resistance in such bacteria, especially *E. coli* may reveal the prevalence of resistance in different animal populations and detect possible transfer of resistant bacteria from animals to humans and vice versa.(1)

For *E. coli* and other classes of enterobacteriaceae in which asypmtomatic colonization of the intestine usually precedes infection, acquisition of one or several new genes, rather than point mutation in existing genes has been shown to be the common ant-microbial resistance mediation route.(4-5) For example, segments of new genes could be replaced with alleles having entirely different sequences from the new type at multiple positions. These new mechanisms thus arise and spread in the bacterial population under conditions of anti-microbial selective pressure.(5)

Again, it has been shown that exposure of most *E. coli* strains to anti-microbial agents usually occurs during treatment directed at infections caused by other unrelated organisms. Anti-microbial treatment that alters the population of *E. coli* in a given host or environment, will usually affect bacterial contacts of the host or environment.(6) Thus, use of a particular antibiotic in for example human hosts, in an environment may increase the risk of colonization by or infection with resistant organisms in other humans or even animals that have not received that set of antibiotics but are sharing common environment with the humans.(5) Anti-microbial use may also increase the density of resistant organisms within a host that already harbor such organisms at a lower density resulting in enhanced shedding of these organisms and increased risk to other hosts.(7)

There is emerging evidence that anti-microbial use in humans may be the major selective force for multi-drug resistant clones in enterobacteriaceae in many developing countries that has resulted in an increasingly high prevalence of multi-drug resistance in these countries.(8-11) This is contrary to the information from the developed countries where overwhelming evidence seems to suggest that anti-microbial use in agriculture is the major driving force in the selection and dissemination of bacterial resistance.(12-17) There is however the need to investigate this further in order to generate sufficient data that could advice on proper use of anti-microbial agents in agriculture in the developing countries.

The present study determines the anti-microbial resistance profile of commensal *E. coli* isolated from free range chickens in Imo state, Nigeria.

Materials And Methods

Study Area:

The study was carried out in Imo State, Nigeria. Imo State is situated in the central part of the southeastern region of Nigeria. The State is divided into 27 Local Government Areas (LGA) for administrative purposes. These LGAs are further grouped into 3 senatorial zones namely, Owerri, Orlu and Okigwe. The agro-ecological characteristics and poultry production systems in the area have been reported.(18)

Identification and selection of sampling sites: The study was conducted in September 2003 at 3 purposively selected peri-urban sites spread across the 3 senatorial zones of the state. The sites included Nekede (Owerri senatorial zone), Amaraku (Okigwe senatorial zone) and Umuaka (Orlu senatorial zone). A preliminary field investigation was made by the researchers to identify the study sites and to make themselves known to the selected farm operators, and discuss the detailed nature of the work with them.

At each site, the families that own the chicken and the number of chicken to be sampled were determined according to the method previously described by Okoli.(19) Each sampling site was visited twice over a period of three weeks. It was determined that the birds have not received any antibiotic medication in the previous two months, since antibiotic treatment has been shown to compromise resistance results.(20) Altogether, samples were collected from local chicken, free-range exotic cockerels, old layers, turkeys and broiler roosters.

Collection of samples, cultivation and isolation of organisms: Cloacae swabs were collected from at least 5 birds randomly selected from the free-range flock at each study site, using sterile swab sticks (AntecR). MacConkey agar (MCA) (Fluka BioChemicaR) was used for selective growth and elucidation of colony characteristics of *E. coli*.(21) The agar was prepared according to manufacturer's instruction and each cloacae swab sample streaked directly on MCA and incubated overnight at 37°C. In all cases, the streaking technique described by Cruickshank et al. (22) was adopted.

After overnight incubation, growths on the MCA plates suggestive of *E. coli* colonies 2-4mm in diameter, opaque and convex with entire edge and rose pink on account of lactose fermentation were further streaked onto eosin methylene blue (EMB) and incubated overnight at 37°C again. Green metallic sheen colonies indicative of *E. coli* were then subjected to biochemical tests, which included indole, methyl red and Simmons citrate tests for *E. coli* identification as described by Edwards and Ewing.(23)

Susceptibility testing: The isolated *E. coli* were screened for anti-microbial resistance profile using the disc diffusion method (24) according to the methods recommended by the National Committee for Clinical Laboratory Standards Guidelines.(25) This was done by streaking the surface of nutrient agar plates uniformly with the organisms and thereafter exposing them to discs (Poly-Tes LabR) impregnated with known concentrations of anti-microbial substances.

Commercial antibiotics discs used in the study were 10 and included AM, ampicillin (25ug); CO, cotrimoxazole (50ug); NI, nitrofurantoin (100ug); GN, gentamycin (10ug); NA, nalidixic acid (30ug); TE, tetracycline (30ug); CH, chloramphenicol (10Ug); CF, cefuroxime (20ug); NB, norfloxacin (10ug); CP, ciprofloxacin (5ug).

Interpretations and data treatment: Susceptibility data were recorded quantitatively by measuring the diameters to the nearest whole millimeter using a meter rule. Following the interpretative chat of the Kirby-Bauer Sensitivity Test Method (26), the zones were interpreted as resistant or sensitive. For the purpose of the present study, isolates with intermediate sensitivity were categorized as sensitive. Furthermore, proportions of isolates resistant to individual drugs and having each anti-microbial resistance patterns were computed as averages and percentages across the different poultry types.

Results

Anti-microbial Resistance Profile of E. coli *Isolates from Local Chicken:*

The overall mean percentage resistance figures of *E. coli* strains isolated from local fowls is shown in Figure 1. Among these, ampicillin and co-trimoxazole were resisted 100 and 78.9% of the time respectively while ciprofloxacin, gentamicin and norfloxacin recorded 0.0, 5.3 and 5.3% resistance respectively.

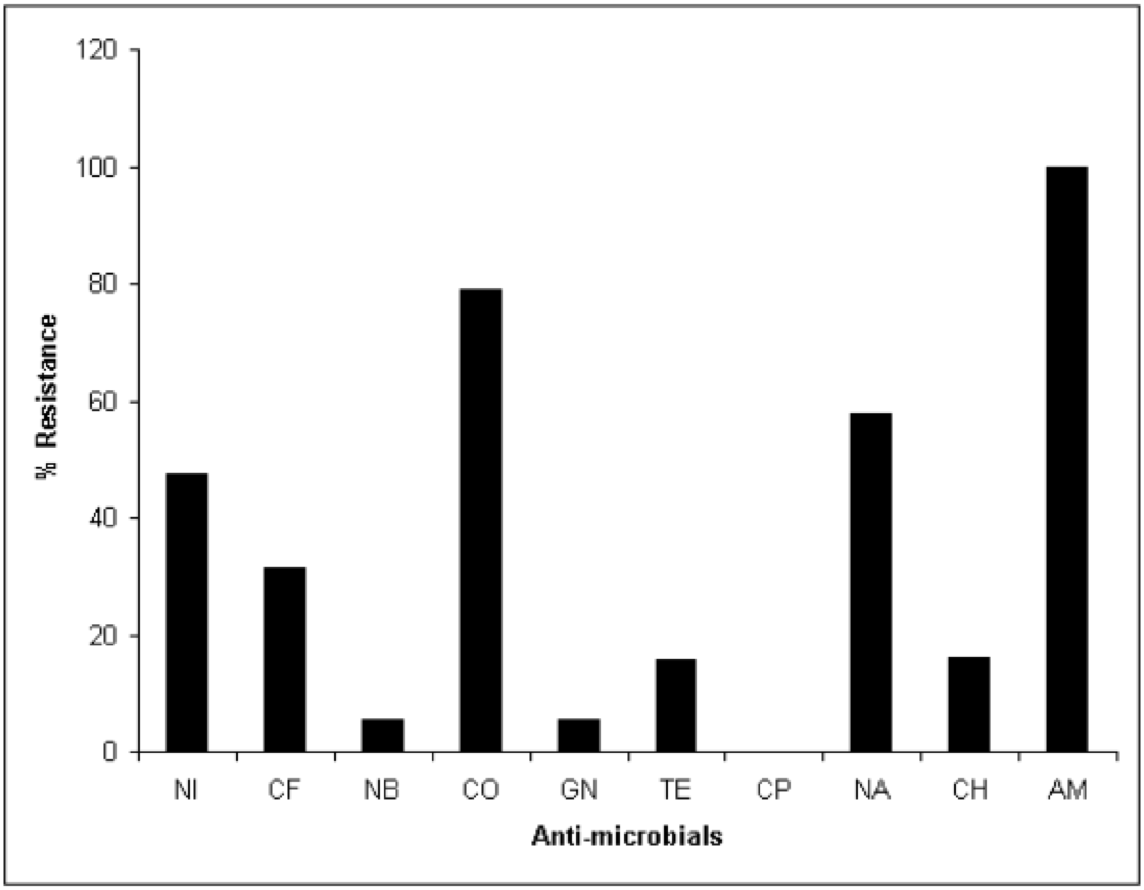

Figure 1: Anti-microbial resistance frequencies of *E. coli* isolates from local fowls reared at different rural locations. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Anti-microbial Resistance Profile of E. coli Isolates from Free-Range Cockerels: Among the free-range-cockerels (Figure 2), *E. coli* isolates recorded 100% resistance against norfloxacin, cotrimoxazole and ampicillin, while for nitrofurantoin it was 83.3% resistance. Gentamicin, tetracycline and ciprofloxacin on the other hand recorded zero resistance.

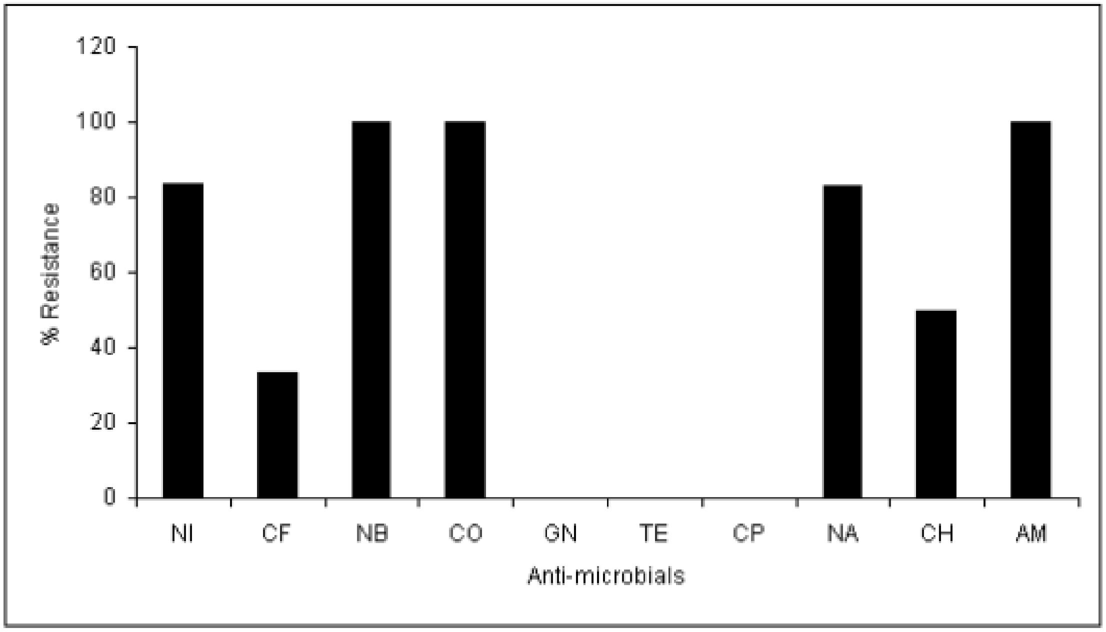

Figure 2: Anti-microbial resistance frequencies of *E. coli* from free range cockerel. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Anti-microbial Resistance Profile of E. coli *Isolates Old Layers: E. coli* isolates from the old layers, (Figure 3), recorded 100% resistance against nitrofurantoin, cotrimoxazole, tetracycline, chloramphenicol and ampicillin, while 80% resistance was recorded against nalidixic acid. Zero resistance was on the other hand recorded against gentamicin.

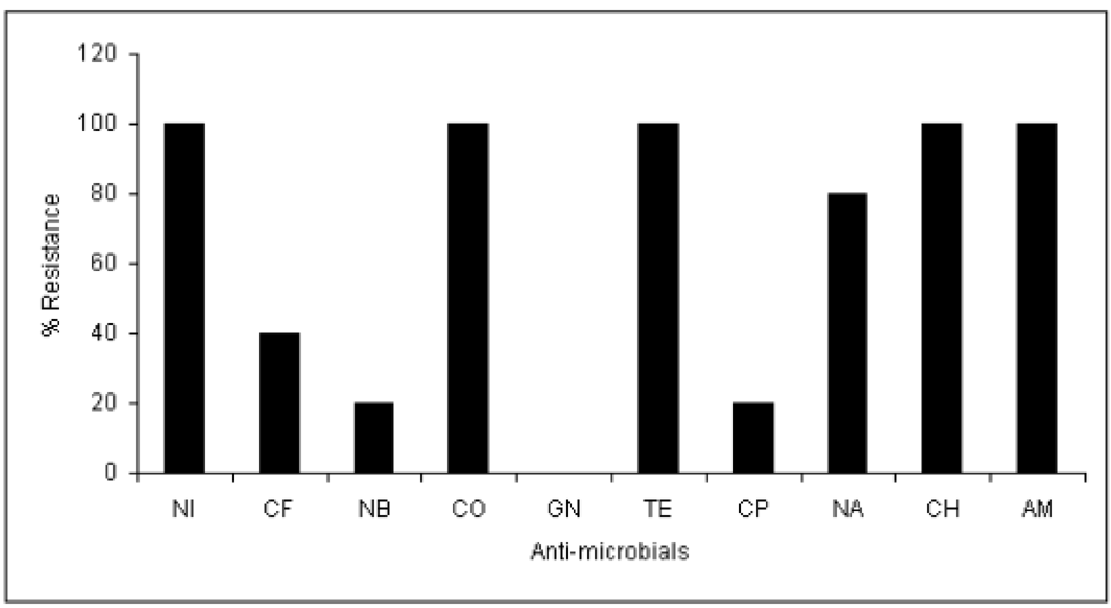

Figure 3: Anti-microbial resistance frequencies of *E. coli* from old layers. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Anti-microbial Resistance Profile of E. coli *Isolates from Free-Range Turkey:* Figure 4 shows that free-range turkeys yielded *E. coli* organism that were highly resistant to co-trimoxazole, nalidixic acid and ampicillin (100%), and nitrofurantoin and chloramphenicol (66.7%). These organisms however recorded 0% resistance to gentamicin and ciprofloxacin.

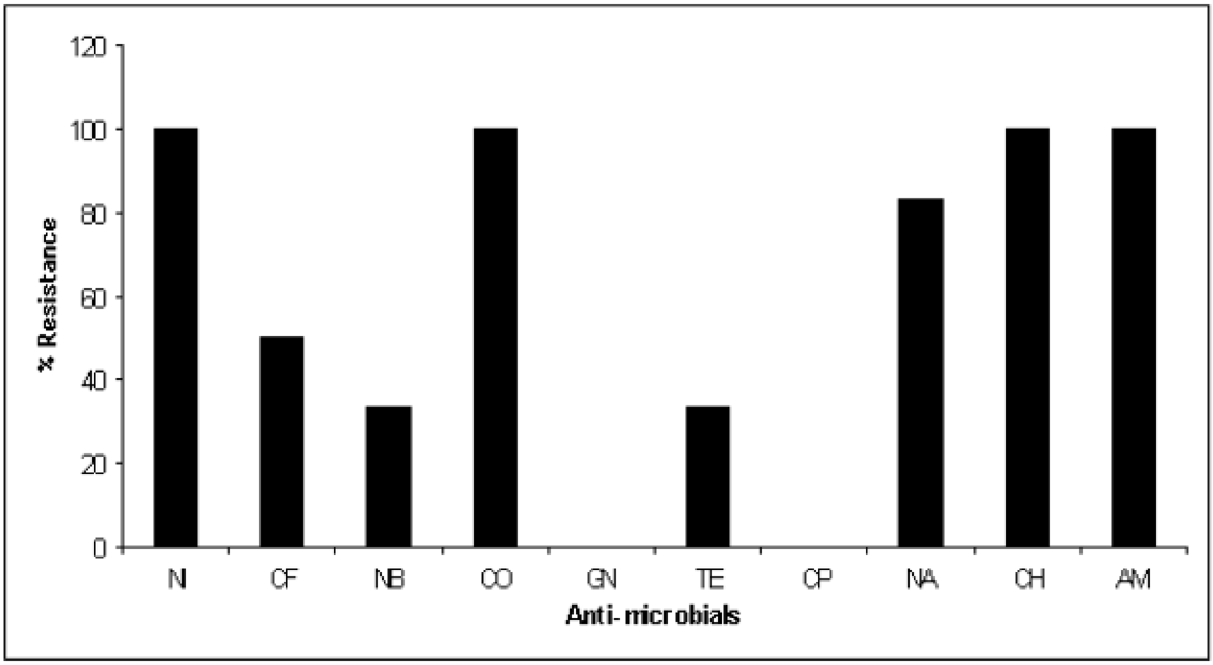

Figure 4: Anti-microbial resistance frequencies of E coli isolates from turkey. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Anti-microbial Resistance Profile of E. coli *Isolates from Broiler Roosters: E. coli* isolates from broiler roosters (Figure 5) recorded 100% resistance against cotrimoxazole and ampicillin, 85.7% to nalidixic acid and chloramphenicol and 78.6% to nitrofurantoin. Furthermore, gentamicin, ciprofloxacin and cefuroxime recorded 0, 7.1 and 7.1% resistance respectively.

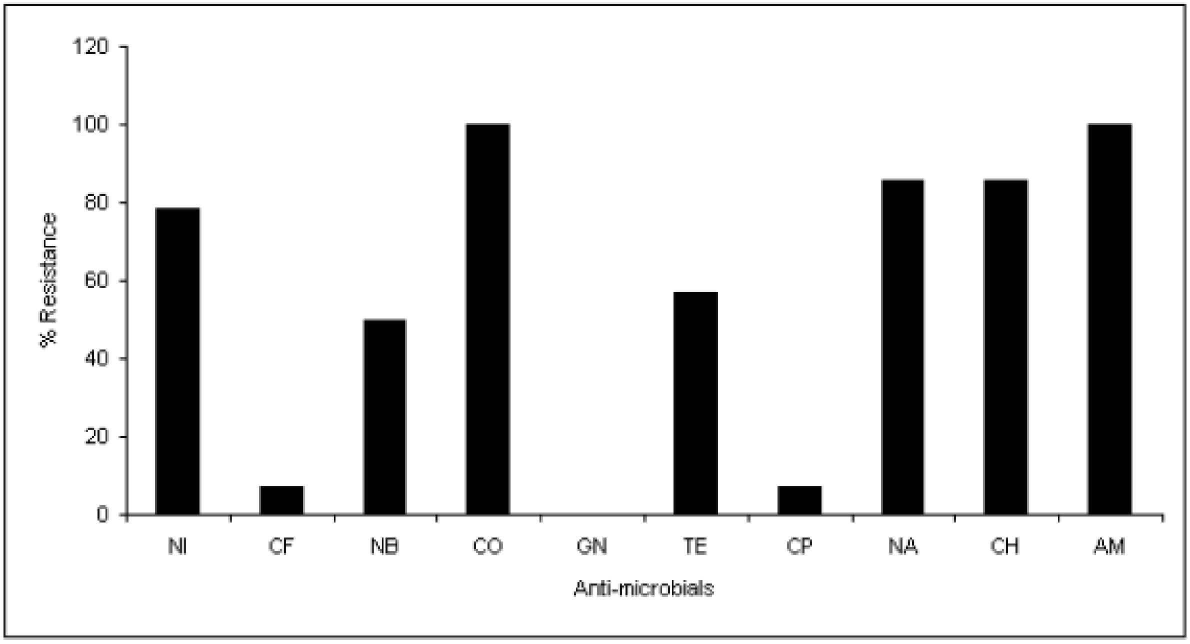

Figure 5: Anti-microbial resistance frequencies of *E. coli* isolates from broiler roosters. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Anti-microbial Resistance Patterns:

E. coli strains isolated from the various free-range poultry types, demonstrated 29 resistance patterns with NI-CO-TE-NA-CH-AM being the most predominant (Table 1). This pattern occurred six times and was elucidated from local fowl, old layers, turkeys and broiler roosters. This was followed by the NI-CO-NA-CH-AM pattern that occurred five times and in local fowls, free-range cockerels and turkeys. Another pattern, NI-NB-CO-TE-NA-CH-AM appeared four times and was observed in old layers and broiler roosters. It was also noted that most of the resistance patterns contained from 5 to 8 antibiotics indicating multi-drug resistance among the organisms.

Table 1: Antimicrobial resistance patterns of *E. coli* isolates from free range poultry in Imo state.

Resistance patterns	Frequency of Dissemination	Occurrence patterns
1 AM		
2 NI-AM		
3 CO-AM		
4 CF-CO-AM	2	LF, FRC
5 CO-NA-AM	2	LF
6 NI-CO-AM		
7 CO-CH-AM	2	LF, RO
8 CO-NA-CH-AM	3	LF, RO
9 NI-CF-CH-AM		
10 NI-CO-NA-AM		
11 CO-GN-NA-CH-AM		
12 NI-CF-CO-TE-AM		
13 NI-CO-NA-CH-AM	5	LF,FRC, TK
14 NI-CF-NA-CH-AM		
15 NI-CF-CO-NA-AM		
16 NI-CO-TE-CH-AM		
17 NI-NB-CO-NA-AM		
18 NI-CO-CP-NA-AM		
19 CF-CO-NA-CH-AM		
20 CF-CO-TE-NA-CH-AM		
21 NI-CF-CO-NA-CH-AM		
22 NI-NB-CO-NA-CH-AM	3	LF, TK, RO
23 NI-CO-TE-NA-CH-AM	6	LF,OL,TK, RO
24 NI-CF-CO-TE-CH-AM		
25 NI-NB-CO-TE-CH-AM		
26 NI-CF-CO-TE-NA-CH-AM	2	OL, TK
27 NI-NB-CO-TE-NA-CH-AM	4	OL, RO,
28 NI-CF-CO-TE-CP-NA-CH-AM		
29 NI-CF-NB-CO-TE-NA-CH-AM	2	TK, RO

Discussion

E. coli isolates from local fowls that rarely receive antibiotics recorded very high resistance against ampicillin and cotrimoxazole, and moderate rates to nalidixic acid and nitrofurantoin. These figures are at variance with the much lower figures reported for ampicillin and cotrimoxazole in local chicken at Nsukka, Enugu state, Nigeria.(27) The high figures presented here may not be out of place but a reflection of resistance events in other hosts sharing the same environment with the local fowls. Although local fowls hardly receive any modern veterinary attention, they may maintain close contact through a myriad of routes with organisms originating from other important hosts in their environment such as humans and exotic chicken that had been previously exposed to various antibiotics. For example, in many rural communities in southeastern Nigeria, it is common for people to defecate in and around surrounding compound bushes or to urinate just at the corner of the house.

Such poor and unhygienic disposal methods of human excrements definitely expose local fowls that feed on such excrement to normal human enteric flora that may harbor novel resistant factors. Furthermore, an enhancement of risk for acquisition of resistant organisms by animal hosts, because of selective use of antibiotics in other hosts in the same environment has recently been described by Lipsitch and Samore.(5)

Resistance events in *E. coli* isolates from free-range cockerels exceeded that of local fowls. It would seem that their exposure to resistance trends in the commercial farms where they were started continued to influence events in colonizing organisms even at their definitive locations. Thus, ampicillin, cotrimoxazole, nitrofurantoin, norfloxacin and nalidixic acid were highly resistant in this group. The 100% resistance returned by the *E. coli* isolates to norfloxacin is of public health importance. This probably the first documented evidence of such a very high resistance figure against any fluoroquinolone in bacterial isolates from farm animals in Nigeria. In fact, Chah et al. (27) reported the highest resistance rate of 27.7% to norfloxacin in *E. coli* from broilers. Ciprofloxacin, which is the only other fluoroquinolone tested in this group however, recorded 0% resistances in the *E. coli* isolates.

It is probable that even though norfloxacin and ciprofloxacin belong to the first generation fluoroquinolones, the latter may have been introduced first into clinical practice in the country. Both medicines however remain unavailable for veterinary prescription in Nigeria. Current information indicates that quinolone resistance can also be plasmid mediated, involving the qnr gene, which is quite distinct from the known quinolone resistance genes. The gene has been isolated from *E. coli* and *Klebsiella pneumoniae* and has been identified in USA and China.(28) It is probable that this plasmid-mediated gene may be contributing to the wide distribution of high bacterial resistance to quinolones in the study area.

The resistance rates observed in *E. coli* isolates from the old layers are alarming. One hundred percent resistance was recorded against nitrofurantoin, cotrimoxazole, tetracycline, chloramphenicol and ampicillin, and 80% for nalidixic acid indicating that these compounds have become seriously compromised and probably are currently of little value in the treatment of *E. coli* infections in the area. These organisms may also constitute enormous reservoirs for genes encoding resistance against these antibiotics and foci for continual spread of these mechanisms.(29-30) The present figures, although higher are similar to the 93.3, 90.0, 70.0, and 60% resistance to ampicillin, tetracycline, chloramphenicol, nitrofurantoin and cotrimoxazole respectively reported by Chah et al. (20) in colisepticeamic *E. coli* strains at Enugu state and Okoli et al (31) in *E. coli* isolates from a commercial layer poultry farm in Owerri, Imo state, Nigeria.

E. coli isolates from turkeys and broiler roosters returned high figures for the inexpensive broad-spectrum first line antibiotics (ampicillin, nalidixic acid, cotrimoxazole, nitrofurantoin and chloramphenicol), although values varied slightly across poultry types. These results once more tally with those of the other poultry types especially free range cockerels and old layers earlier reported, thus confirming high resistance profiles against these drug to be the order in *E. coli* isolates from different types free-range poultry in Imo state.

Twenty-nine resistance patterns were observed in the E. coli isolates with predominant patterns being distributed widely across poultry types indicating a striking diversity of resistance patterns in the areas. Multi-drug resistance was a common feature in these isolates, highlighting the fact that the resistance genes for these drugs are linked on plasmids.(32) Moreover, the wide spread resistance to cotrimoxazole may be implying the presence of class 1 integrons, which are also important in conferring resistance to multiple anti-microbials.(33)

Norfloxacin was incorporated in many of the patterns observed in the present study especially the predominant ones. This probably points to the fact that the E. coli strains encountered in these free-range poultries might have originated from human hosts where their resistances to fluoroquinolones were first selected and subsequently disseminated to lower animals.(5) There is strong evidence that anti-microbial use in humans has not only driven the emergence of multi-drug resistant clones in the developing countries such as Nigeria that has resulted in an increasingly high prevalence of multiple resistance.(8-11)

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