

Original Article

Anti-Microbial Resistance Profiles Of *E. Coli* Isolated From Free Range Chickens In Urban And Rural Environments Of Imo State, Nigeria

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Abstract:

Information on the resistance profiles of normal intestinal flora of extensively reared chickens that hardly receive antibiotics in the developing countries can serve as important means of understanding the human/animal pathogens drug resistance interactions in the zone. Three hundred and fifty *E. coli* isolates, comprising 133 from urban and 217 from rural sites in Imo state, Nigeria, were screened for anti-microbial resistance profile against 10 antibiotics using the disc diffusion method. Overall percentage anti-microbial resistance of the isolates against cotrimoxazole, ampicillin, nalidixic acid, chloramphenicol and nitrofurantoin (72–92%) were very high. The organisms were highly sensitive to other antibiotics, especially gentamicin and ciprofloxacin. The 59.5% overall mean percentage resistance recorded at the urban area was significantly higher than the 46.8% recorded at the rural area ($p < 0.05$). With the exception of the figures for cotrimoxazole and ampicillin, resistance values obtained against the other antibiotics at the urban sites were statistically higher than those obtained at the rural sites ($p < 0.05$). Zero resistance was recorded against the fluoroquinolones, norfloxacin and ciprofloxacin at all the rural sites except at Enyigwugwu where a 28.6% resistance was obtained against norfloxacin. Since free-range chickens rarely receive antibiotic medication, it is concluded that the highly resistant *E. coli* organisms isolated from them may be reflecting consequences of human drug use in the study areas.

Key Words: Anti-microbial resistance, *E. coli*, free-range chicken, antibiotics, Nigeria

Introduction:

The development of anti-microbial resistance in many bacterial organisms constitutes serious problem in the control of infectious diseases.(1-3) Anti-microbial use and especially misuse has been found to be the most important selecting force in bacterial antibiotic resistance.(4-6) Although there is no linear relationship between the amounts of anti-microbials used and the development of resistance, increased use of an anti-microbial often results in decreased susceptibility among exposed bacteria.

An anti-microbial selective pressure may arise because of cross-resistance and co-selection of resistance genes and may explain how one anti-microbial selects for another anti-microbial. Again, multiple resistances may confer resistance to several anti-microbials, while virulence and lack of hygiene may account for the survival and spread of resistant bacteria, even in the absence of an anti-microbial selection pressure.(7)

The bacteria belonging to the normal intestinal flora of humans and animals, which constitute an enormous reservoir of resistance genes for potentially pathogenic bacteria may serve as major indicators for selection pressure exerted by antibiotic use in a given animal or human population.(8-10) Investigations, especially on *E. coli* and enterococci, make it possible to understand the prevalence of resistance in different animal populations and to detect a possible transfer of resistant bacteria from animals to humans and vice versa.(11) Nijsten *et al.*, (12,13) for example, found significantly more resistant *E. coli* in the faecal flora of pig farmers than in faecal sample of pig slaughterers and urban residents. Other studies have shown that even when an antibiotic was restricted to veterinary use alone, resistance genes may not only be found in animal isolates or zoonotic bacteria isolates from humans, but also from enterobacteriaceae in the environment, the intestinal flora of farmers and hospital isolates.(14-16)

Although the use of anti-microbial drugs in food animals have been shown to lead to resistant strains of pathogens which may be transmitted to humans through food in

the developed countries, the contribution of anti-microbial use in food animals to resistance in bacteria infecting humans or vice versa in developing countries remain unclear. There is however strong evidence that anti-microbial use in humans has not only driven the emergence of multi-drug resistant clones in these countries, but has resulted in an increasingly high prevalence of multiple resistance.(17-19)

There is therefore the need to continue investigating the resistance profiles of normal intestinal flora of extensively reared animals that hardly receive antibiotics in the developing countries as a means of understanding the human/animal drug resistance interaction in the zone. The present study compared anti-microbial resistance profiles of *E. coli* isolates from free-range chickens in urban and rural environments of Imo state, Nigeria.

Imo state is situated in the southeastern rainforest vegetational belt of Nigeria and lies between latitude 5° 4' and 6° 3' N and longitude 6° 15' and 7° 34' E. The agro-ecological characteristics of the area have been reported.(20) Imo 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. Livestock farming especially poultry is popular in these zones. There are usually higher concentrations of poultry farms around major cities.

Poultry production in the state could be broadly divided into extensive, semi-intensive and intensive systems. The greatest population of chicken in the study area is made of local breeds reared by rural farming families under the extensive scavenging system.(21) These producers are quite distinct from the owners of small to medium scale commercial poultry farms that are sited in both rural and urban areas.(22) Rearing of started exotic broilers and cockerels has also become an important aspect of this production system.(23)

Commercial intensive poultry productions in southeastern Nigeria include table eggs, broiler, parent stock, hatchery, turkey and started chicken production. These farming operations are distributed over urban, peri-urban and rural sites and have been shown to range from very small operations (50-100 birds), to medium (101-1000 birds) and large scale (above 1000 birds).(24) Small and medium farms are usually family back yard affairs that are predominantly found in urban and peri-urban centers, while most large scale, operations are located in peri-urban and rural environments.

In most of the back yard poultry, hygienic and bio-security measures are usually poor with all the family members being involved in the daily management activities. Usually there is no organized effort at vermin, ferrets and human traffic control.

Materials and Methods: **Identification and selection of sampling sites**

The study, conducted in September 2003, consisted of three stages, starting with sample collection from the different sites and preceded by a preliminary field investigation, during which the researcher identified the study sites (rural and urban) and made himself known to the farm operators.

For rural environment studies, 6 sites popular for poultry keeping were purposively selected. These included Obibiezena and Obiangwu in Ngor Okpala LGA, Uzoagba in Owerri North, Amaraku in Isiala Mbano, Umuaka in Njaba and Enyiogwugwu in Abor

Mbaise LGAs respectively. Ubomiri in Mbaitolu LGA, Akabo in Ikeduru, Nekede in Owerri West, Orji in Owerri North and Owerri Urban LGAs (5 sites) were selected for the urban environment study. Free range local fowls, layers, cockerels and broiler roosters were sampled at the different sites.

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.(20) 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.(25)

Collection of samples, cultivation and isolation of organisms

Cloacae swabs were collected from at least 5 birds randomly selected from free-range flock at each study site, using sterile swab sticks (Antec^R). MacConkey agar (MCA) (Fluka BioChemica^R) was used for selective growth and elucidation of colony characteristics of *E. coli*.(26) The agar was prepared according to manufacturers 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* (27) 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.(28)

Susceptibility testing

The isolated *E. coli* were screened for anti-microbial resistance profile using the disc diffusion method (29) according to the methods recommended by the National Committee for Clinical Laboratory Standards Guidelines.(30) This was done by streaking the surface of nutrient agar plates uniformly with the organisms and thereafter exposing them to discs (Poly-Tes Lab^R) impregnated with known concentrations of anti-microbial substances.

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

Statistical analysis

Susceptibility data were recorded quantitatively by measuring the diameters to the nearest whole millimeter using a meter rule. Following the interpretative chart of the Kirby-Bauer Sensitivity Test Method (31), 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 sample sites in the urban and rural environments.

Significant differences in mean percentage resistances were determined across the sample sites using the Student t-Test method.(32)

Results:

Overall, 350 *E. coli* isolates comprising 133 from urban and 217 from rural sites and means of 26.6 and 36.2 isolates/site for both sites respectively were obtained (Table 1)

SN	Urban/Peri-urban sites			Rural sites		
	Location	Local Govt. Area	No of iso-lates	Location	Local Govt. Area	No of iso-lates
1	Owerri	Owerri urban	42	Obibiezena	Ngo-Okpala	63
2	Nekede	Owerri west	28	Enyiogwugwu	Abo-Mbaise	49
3	Ubomiri	Mbaitolu	35	Uzoagba	Owerri north	28
4	Orji	Owerri north	14	Obiangwu	Ngo-Okpala	28
5	Akabo	Ikeduru	14	Umuaka	Njaba	28
6				Amaraku	Isiala Mbano	21
Total			133			217
Mean			26.6			36.2

Overall number of *E. coli* isolates = 350

Figure 1 showed that the overall percentage anti-microbial resistance of the *E. coli* isolates against cotrimoxazole, ampicillin, nalidixic acid, chloramphenicol and nitrofurantoin (72–92%) were very high. The other antibiotics, especially gentamicin and ciprofloxacin were highly sensitive to the organisms.

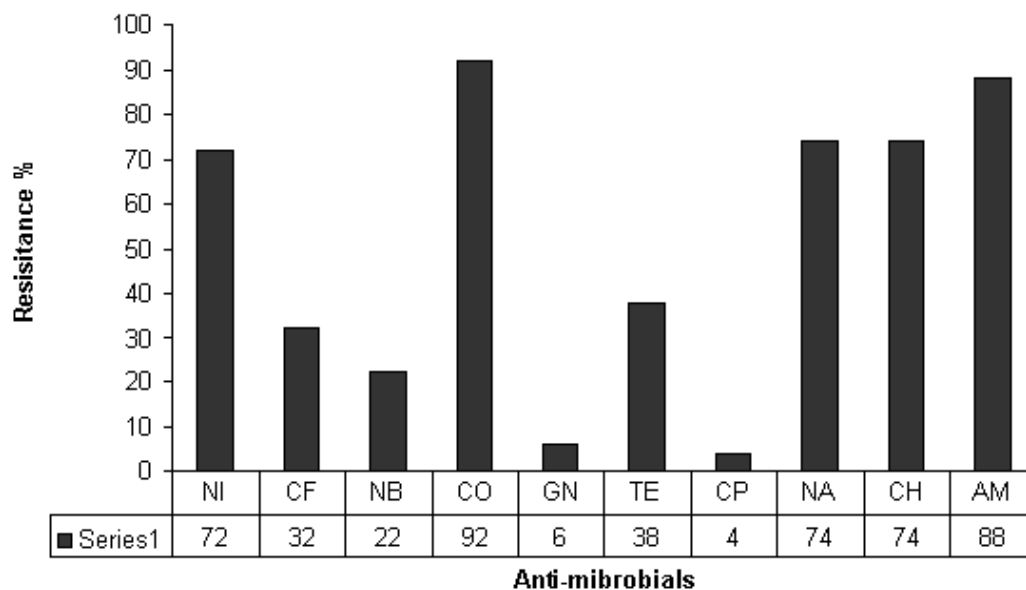


Figure 1: Overall percentage anti-microbial resistances of *E. coli* isolates from urban and rural environments to various anti-microbial agents. NI, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin

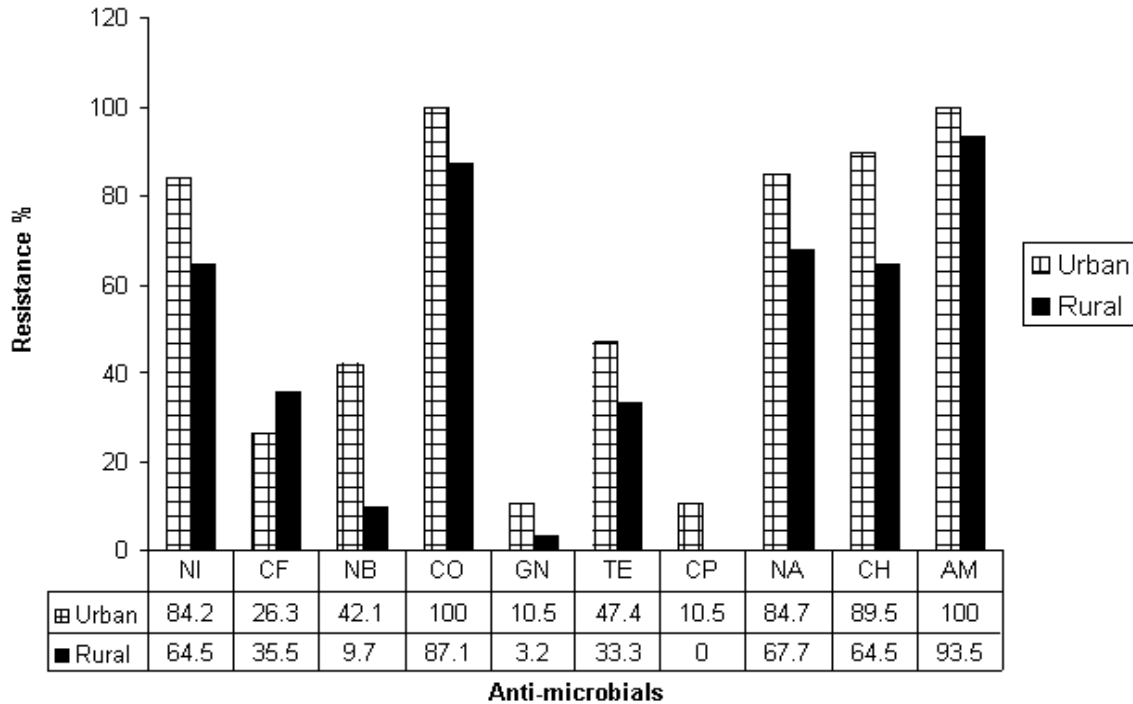


Figure 2: Comparison of overall anti-microbial resistance in *E. coli* isolates from urban and rural environments in Imo state, Nigeria. NI, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin

Figure 2 showed that *E. coli* isolates from the urban areas were more resistant to any of the tested antibiotics than those from rural areas. For example, while zero resistance was recorded against ciprofloxacin in the rural area, 10.5% was obtained in the urban area. Resistance figures however remained high at both sites for nitrofurantoin, cotrimoxazole, nalidixic acid, chloramphenicol and ampicillin. Statistical treatment of these percentage resistance differences at the two study areas (Table 2), revealed that with the exception of cotrimoxazole and ampicillin, antibiotic resistance obtained at the urban areas were generally statistically higher than those obtained at the rural areas ($p < 0.05$). The 59.5% overall mean percentage resistance recorded at the urban area was also significantly higher than the 46.8% recorded at the rural area ($p < 0.05$).

Table 2: Comparison of overall anti-microbial resistance in *E. coli* isolates from urban and rural environments in Imo state, Nigeria.

Anti-microbial	Urban/Peri-urban	Rural	p - value
Nitrofurantoin (NI)	112 (84.2)	140 (64.5)*	.0839
Cefuroxime (CF)	35 (26.3)	77 (35.5)	.0941
Norfloxacin (NB)	56 (42.1)	21 (9.7)	.3558
Cotrimoxazole (CO)	133 (100.0)	189 (97.1)	.0438
Gentamicin (GN)	14 (10.5)	7 (3.2)	.33117
Tetracycline (TE)	63 (47.4)	70 (32.3)	.1192
Ciprofloxacin (CP)	14 (10.5)	0 (0.0)	.5000
Nalidixic acid (NA)	112 (84.2)	143 (67.7)	.0689
Chloramphenicol (CH)	119 (89.5)	140 (64.5)	.1025
Ampicillin (AM)	133 (100.0)	203 (93.5)	.0214
Total number of isolates	133	217	-
Overall mean % resistance	59.5	46.8	.0752

p <0.05; *values in parenthesis are percentages resistance

Across the different urban sites (Table 3), resistance against the nitrofurantoin, cotrimoxazole, nalidixic acid, chloramphenicol and ampicillin remained generally high, while for tetracycline, similar high resistance figures were recorded at Owerri, Nekede and Ubomiri. While zero resistances were recorded at gentamicin at all the sites, an abnormally high (100%) resistance was obtained at Orji. Again, moderately high (40 – 50%) resistances were recorded for norfloxacin at Owerri, Nekede and Ubomiri, while at Akabo, 50% resistance was also recorded against ciprofloxacin.

Table 3: Anti-microbial resistance frequencies of *E. coli* isolates from urban environments in Imo state.

Site	NI	CF	NB	CO	GN	TE	CP	NA	CH	AM	n
Owerri	35(83.3)	7(16.7)	21(50.0)	42(100.0)	0(0.0)	21(50.0)	0(0.0)	35(83.3)	42(100.0)	42(100.0)	42
Nekede	21(75.0)	7(25.0)	14(50.0)	28(100.0)	0(0.0)	14(50.0)	0(0.0)	28(100.0)	28(100.0)	28(100.0)	28
Ubomiri	28(80.0)	14(40.0)	14(40.0)	35(100.0)	0(0.0)	28(80.0)	7(20.0)	28(80.0)	35(100.0)	35(100.0)	35
Orji	14(100.0)	7(50.0)	0(0.0)	14(100.0)	14(100.0)	0(0.0)	0(0.0)	7(50.0)	14(100.0)	14(100.0)	14
Akabo	14(100.0)	0(0.0)	7(50.0)	14(100.0)	0(0.0)	0(0.0)	7(50.0)	14(100.0)	0(0.0)	14(100.0)	14
Total	112(84.2)	35(26.3)	56(42.1)	133(100.0)	14(10.5)	63(47.4)	14(10.5)	112(84.2)	119(89.5)	133(100.0)	133

NI, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamicin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin

Across the rural sites (Table 4), resistance against nitrofurantoin, cotrimoxazole, nalidixic acid, chloramphenicol and ampicillin were again generally high with exception of the zero resistance recorded against chloramphenicol at Uzoagba and the 25% recorded against nitrofurantoin at Obibiezena and Uzoagba. An abnormally high 100% resistance was equally recorded against gentamicin at Obibiezena while at the other sites resistance was generally zero. Zero resistances were recorded against norfloxacin and ciprofloxacin at all the sites except at Enyigwugwu where a 28.6% resistance was obtained against norfloxacin.

