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Antimicrobial resistance of non-clinical *Escherichia coli* strains from chicken in Nsukka, South-east Nigeria

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Abstract

This study was carried out to determine resistance profiles of Escherichia coli strains isolated from clinically healthy chickens in Nsukka, southeast Nigeria. A total of 324 E. coli strains isolated from cloaca swabs from 390 chickens were tested against 16 antimicrobial agents using the disc diffusion method. The antibiotics used in the study were: ampicillin (25µg), amoxycillin-clavulanic acid (30µg), gentamicin (10µg), streptomycin (30µg), cefuroxime (20µg), cephalexin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), norfloxacin (10µg), ofloxacin (5µg), pefloxacin (5µg), tetracycline (30µg), chloramphenicol (10µg), cotrimoxazole (50µg), colistin (25µg) and nitrofurantoin (100µg). The strains demonstrated high rates of resistance (34.6% - 66.1%) to ampicillin, tetracycline, nitrofurantoin, cefuroxime and cotrimoxazole. None of the isolates was resistant to colistin, ofloxacin and pefloxacin. For each antimicrobial agent (except cephalexin), strains from the intensively reared chickens (layers and broilers) displayed higher resistance frequencies than those from the local birds. A total of 49 resistant patterns were recorded for the 228 strains resistant to at least one antimicrobial drug, with AmTeCoS and AmTeCfN being the predominant patterns.
Because of the great variation in the drug resistance patterns of the Escherichia coli strains, use of antimicrobial agents in the management of E. coli infections in the study area should be based on results of sensitivity tests.

Key Words: Antimicrobial, resistance, patterns, *Escherichia coli*, chicken.

Introduction

Antimicrobial resistance had been recognized since the advent of antimicrobial agents. However, the consequences of the emergence were dramatically controlled by the continued availability of effective alternatives (Neu, 1992). Unfortunately, at the moment, drug resistance poses a serious threat to antibiotic therapy due to the growing number of pathogens resistant to multiple, structurally unrelated drugs and the slow pace in developing new antimicrobials (Neu, 1992). White *et al.*, (1997) pointed out that the emergence of resistant bacteria has been and continues to be of concern to clinicians, public health officials and clinical microbiologists.

Van den Bogaard (1993) suggested that one of the measures that must be taken to prevent emergence/spread of antimicrobial resistance was a systematic registration and analysis of patterns of bacteria resistance in pathogenic and non-pathogenic faecal flora. Lens (1993) also indicated that *in vitro* surveillance of drug resistance should be part of a package of information required on a routine basis if the best possible use of antibiotics and a reduction of induction of resistance are to be achieved. The seriousness of the problems posed by the evolution and dissemination of multi-drug resistant bacteria is reflected by the existence of many international and national network programmes involved in the surveillance of antimicrobial resistance in bacteria. For

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example, the Reservoir of Antibiotic Resistance (ROAR), an international network programme established by the Alliance for the Prudent Use of Antibiotic (APUA), is concerned with the generation of information on commensal bacteria that serve as reservoirs of transferable drug resistance genes. Also, in many European countries, national antimicrobial monitoring programmes (such as DANMAP and FIRE Works in Denmark & Finland respectively) have been set up by the respective governments to monitor antimicrobial resistance in pathogenic and non-pathogenic human and animal bacterial species.

In much of the developing world, antimicrobial susceptibility profiles of bacterial isolates are unknown (Okeke *et al.*, 1999). In southeast Nigeria, drug resistant colisepticaemic *E. coli* strains were found to constitute a serious threat to poultry production (Chah and Oboegbulem, 1998; Chah *et al.*, 2000). In the region treatment of suspected bacterial infections is based largely on "experience". Lack of veterinary diagnostic laboratories and unwillingness by the farmers to pay for cost of laboratory diagnosis were identified as the major reasons impeding isolation, identification and sensitivity testing of bacterial agents from animal sources prior to antimicrobial therapy (Chah and Nweze, 2001). John and Fishman (1997) pointed out that routine susceptibility testing of bacterial isolates and surveillance of antibiotic resistance, which provides information on resistance trends including emerging antibiotic resistance are essential for clinical practice. Thus, as part of the global efforts to curtail the evolution and dissemination of drug resistant bacterial species, this study was initiated to determine resistance profiles of non-clinical *E. coli* strains from chickens in Nsukka, southeast Nigeria. The results will provide baseline information on antimicrobial resistance among *E. coli* isolates in the study area and will also act as a guide for empirical antimicrobial therapy in cases of avian *E. coli* infections.

Materials and methods

Cloaca swabs from clinically healthy exotic chickens in poultry flocks (flock size ranged from 300 to 2000 birds) and clinically healthy local (native) chickens reared in some households in Nsukka, southeast Nigeria were used in this study. The swab samples collected from a total of 390 (180 layers, 150 broilers and 60 local) chickens were cultured not later than 2 hrs after collection.

Each swab was streaked on MacConkey agar plate and incubated at 37°C overnight. From each plate, a single rose-pink colony was selected and streaked on eosin-methylene blue (EMB) agar and incubated overnight at 37°C. Greenish-metallic sheen colonies on EMB were further subjected to biochemical tests (Indole, Methyl Red, Voges-Proskauer and Simmons citrate) for *E. coli* identification as described by Edwards and Ewing (1972). Identified *E. coli* isolates were screened for antimicrobial resistance profile using the disc diffusion method (Bauer *et al.*, 1966). Each *E. coli* isolate was grown in nutrient broth at 37°C for 4 hours. About 0.1ml of the 4hr broth cultures was spread on Mueller-Hinton agar. The inoculated plate was allowed to dry for 30 minutes before the antimicrobial discs (8 discs per plate) were applied on its surface. The plates were allowed on the bench for 30 minutes before they were inverted and incubated at 37°C for 16-18 hours. The diameter of the zones of inhibition were measured with a metre rule and recorded to the nearest whole millimetre. The zones were interpreted as resistant or sensitive following the interpretative chart of the Kirby-Bauer Sensitivity Test method (Cheesbrough, 2000). Commercial antimicrobial discs used in the study included: ampicillin (25g), amoxycillin-clavulanic acid (30µg), gentamicin (10µg), streptomycin (30µg), cefuroxime (20µg) cephalexin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), norfloxacin (10µg), ofloxacin (5µg), and pefloxacin (5µg)], tetracycline (30µg), chloramphenicol (10µg), cotrimoxazole (50µg), colistin (25µg) and nitrofurantoin (100µg).

Results

A total of 324 *Escherichia coli* strains were isolated from the 390 chickens sampled. The

frequency of resistance of the bacterial strains to the 16 antimicrobials is presented in Table 1.

Table 1 *Frequency of antimicrobial resistance of non–clinical avian E. coli strains*

Antibiotic	No. (%) of resistant strains			
	Layers (n = 162)	Broilers (n = 112)	Local chickens (n = 50)	Total (n = 324)
Ampicillin (25µg)	112 (69.1)	88 (78.6)	14 (28)	214 (66.1)
Tetracycline (30µg)	81 (50.0)	90 (80.4)	4 (8)	175 (54.0)
Nitrofurantoin (100µg)	73 (45.1)	61 (54.5)	5 (10)	139 (42.9)
Cefuroxime (20µg)	58 (35.8)	48 (42.9)	0 (0)	125 (38.6)
Cotrimoxazole (50µg)	54 (33.3)	58 (51.8)	0 (0)	112 (34.6)
Streptomycin (30µg)	12 (7.4)	78 (69.6)	4 (8)	94 (29.0)
Norfloxacin (10µg)	13 (8.0)	31 (27.7)	4 (8)	48 (14.8)
Gentamicin (10µg)	28 (17.3)	12 (10.7)	0 (0)	40 (12.4)
Amoxycillin-clavulanic acid (30µg)	26 (16)	9 (8.0)	4 (8)	39 (12.0)
Chloramphenicol (10µg)	22 (13.6)	8 (7.1)	2 (4)	32 (9.9)
Nalidixic acid (30µg)	14 (8.6)	16 (14.3)	2 (4)	32 (9.9)
Ciprofloxacin (5µg)	4 (2.5)	3 (2.7)	1(2)	8(2.5)
Cephalexin (10µg)	0 (0)	0 (0)	6 (12)	6 (1.9)
Colistin (25µg)	0 (0)	0 (0)	0 (0)	0 (0)
Ofloxacin (5µg)	0 (0)	0 (0)	0 (0)	0 (0)
Pefloxacin (5µg)	0 (0)	0 (0)	0 (0)	0 (0)

The strains displayed high rates of resistance (34.6% - 66.1%) to ampicillin, tetracycline, nitrofurantoin, cefuroxime and cotrimoxazole. Resistance rates to streptomycin, gentamicin, norfloxacin and amoxycillin-clavulanic acid were moderate (12%-29%) while low rates (1.9% - 9.9%) were recorded for chloramphenicol, cephalexin, nalidixic acid and ciprofloxacin. None of the isolates was resistant to colistin, ofloxacin and pefloxacin. Isolates from broilers demonstrated higher rates of resistance to each of the antibiotic than those from layers, except for amoxycillin-clavulanic acid, chloramphenicol and gentamicin where the reverse was the case. Of the 324 *E. coli* isolates tested, 96 (29.6%) were not resistant to any of the drugs while 38 (11.7%), 28 (8.6%) and 162 (50%) strains were resistant to one, two and more than two antimicrobials respectively (Table 2).

Table 2 *Number of antimicrobial agents to which E. coli strains were resistant*

No. of drugs resistant to	No. (% of strains resistant)			
	Broilers	Layers	Local chickens	Total
0	10 (10.4*)	54 (56.3)	32 (33.3)	96 (29.6**)
1	0 (0.0)	28 (73.7)	10 (26.3)	38 (11.7)
2	8 (28.6)	18 (64.3)	2 (7.1)	28 (8.6)
>2	94 (58.0)	62 (38.3)	6 (3.7)	162 (50)

* = % of row total

** = % of total number of E. coli strains isolated (324)

Of the 28 strains resistant to two antibiotics only two were from the local (native) chickens while 8 and 18 were from the broilers and layers respectively. Also, of the 162 strains resistant to more than two antibiotics 6, 62 and 94 were isolated from the local birds, layers and broilers respectively. A total of 49 resistant patterns were recorded for the 228 strains resistant to at least one antibiotic drug, with AmTeCoS and

AmTeCfN being the predominant patterns (Table 3).

Table 3 *Antimicrobial resistance patterns of non-clinical E. coli strains*

Resistance patterns	No. (%) of strains
Am	14 (4.86)
Te	4 (1.39)
Nb	2 (0.69)
Co	4 (1.39)
Au	8 (2.78)
Na	2 (0.69)
Cf	2 (0.69)
Cx	2 (0.69)
Am+Te	2 (0.69)
Am+Cx	2 (0.69)
Am+Co	4 (1.39)
Am+Cf	6 (2.08)
Am+N	2 (0.69)
Te+N	4 (1.39)
Te+Cf	2 (0.69)
Cf+N	4 (1.39)
Co+Cf	2 (0.69)
Am+Te+S	2 (0.69)
Am+Te+Cf	6 (2.08)
Am+Te+Co	8 (2.78)
Am+Cf+N	6 (2.08)
Am+Co+N	8 (2.78)
Am+G+N	2 (0.69)
Am+Cf+Co	2 (0.69)
Am+Co+Au	2 (0.69)
Am+Co+Na	2 (0.69)
Te+Cf+N	6 (2.08)
Te+Co+Cf	2 (0.69)
G+Co+Cf	2 (0.69)
Am+Te+Cf+N	22 (7.64)
Am+Te+Co+Cf	4 (1.39)
Am+Te+Co+S	28 (9.72)
Am+Na+Cf+N	2 (0.69)
Am+Na+Te+Co	2 (0.69)
Am+G+Co+N	8 (2.78)
Am+Cx+Au+Na	2 (0.69)
Am+Na+Te+Cf+N	4 (1.39)
Am+Te+Co+Cf+N	10 (3.47)
Am+C+G+Cf+N	2 (0.69)
Am+Te+G+Co+N	2 (0.69)
Am+C+Te+G+Na	2 (0.69)
C+Te+Co+Cf+N	2 (0.69)
C+G+Co+Nb+Cf	2 (0.69)
Am+Na+Te+Co+Nb+N	2 (0.69)
Am+Na+Te+Nb+Cf+N	2 (0.69)
Am+C+G+Co+Cf+N	4 (1.39)
Am+Na+Cp+G+Co+Nb+Cf+N	2 (0.69)
Am+C+Na+Cp+Te+Co+Cf+N	2 (0.69)
Am+C+Na+Cp+G+Co+Nb+Cf+N	4 (1.39)

Am = Ampicillin, Au = Amoxycillin-clavulanic, G = Gentamicin, S = Streptomycin, Cf = Cefuroxime, Cx = Cephalexin, Na = Nalidixic acid, Cp = Ciprofloxacin, Nb = Norfloxacin Te = Tetracycline, C = Chloramphenicol, Co = Cotrimoxazole, N = Nitrofurantoin

Discussion

The non-clinical avian *E. coli* strains in this study demonstrated high rates of resistance against ampicillin, tetracycline and nitrofurantoin. However, these resistance rates are lower than those previously reported (Chah and Oboegbulem, 1998; Chah *et al.*, 2000). This disparity may be attributed to the fact that in our previous reports clinical *E. coli* strains were used unlike in the present study. Drug preparations containing ampicillin, tetracycline or nitrofurantoin are widely used for prophylaxis, growth promotion and treatment of bacterial infections in chickens in southeast Nigeria (Chah and Nweze, 2001). Reports have shown that oral administration of antibiotics at subtherapeutic levels leads to an increase in the incidence of resistant bacteria in the intestines of animals exposed to these drugs (Levy, 1991, Helmuth and Protz, 1997; WHO Report, 1997; Witte, 1998; Witte *et al.*, 1999). Thus, the high resistance rates reported in this study as well as those of other previous works (Chah and Oboegbulem, 1998; Chah *et al.*, 2000) in the area may be attributed to irrational use of antimicrobial agents in poultry production This view is also supported by the fact that isolates from local chickens (where antimicrobials are rarely used) demonstrated low rates of resistance to the antimicrobial agents tested. The large number of resistance patterns recorded in this study suggest that several selective pressures may be involved in the induction of drug resistance among the *E. coli* strains.

Presence in poultry flocks of non-clinical *E. coli* strain with high rates of resistance to commonly available antimicrobial agents in the area poses a serious threat to the veterinarians, farmers and farm workers because they represent a 'gene pool' from which drug resistant infections might arise. Conjugal transfer of drug resistance determinants in bacteria has been reported to occur over species and genus borders (Teuber *et al.*, 1996). Transfer of drug resistance factors to avian pathogens such as *Salmonella spp* and septicaemic *E. coli* strains will render avian infections caused by these pathogens difficult to treat with the commonly available antimicrobial agents. The non-pathogenic resistant *E. coli* may colonize the human intestines where it transfers its resistance factor (R - factor) to the human pathogen or other indigenous flora.

Conclusion

The results of this study and those of several previous reports (Amara *et al.*, 1995; Blanco *et al.*, 1997; Chah and Oboegbulem, 1998; and Chah *et al.*, 2000) indicate an increasing incidence of multiple antibiotic resistance among both pathogenic and non-pathogenic *E. coli* strains. This has largely been attributed to indiscriminate use of antimicrobial agents in animal production (Blanco *et al.*, 1997; Helmuth and Protz, 1997; WHO Report, 1997; Witte *et al.*, 1999). Since a large proportion of the isolates were resistant to ampicillin and tetracycline, it is recommended that the use of such drugs should only be based on results of sensitivity tests. For the treatment of avian *E. coli* infections in southeast Nigeria, the use of drug preparations containing colistin or the fluoroquinolones such as pefloxacin, ofloxacin and ciprofloxacin is suggested. However, as pointed out by Blanco *et al.* (1997), the indiscriminate use of these fluoroquinolones in animals should be avoided as this may lead to cross-resistance with other human enteric pathogens such as *Salmonella* and *Campylobacter spp*. To check the evolution and dissemination of antimicrobial resistant bacteria in the environment, there is an urgent need to establish nationally coordinated antibiotic resistance monitoring programmes in the country. The fundamental objectives of such programmes will be to evaluate and report on a regular basis, trends on antibiotic resistance in animal and human bacteria and factors influencing the observed trends. Such reports should be made available to prescribers and users of antimicrobials and also used to educate the general public on the dangers of irrational use of drugs in humans and animals.

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