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Extended-spectrum beta-lactamase-producing *Escherichia coli* in chickens from small-scale (backyard) poultry farms in Maiduguri, Nigeria

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Abstract

Aim: This study investigated the occurrence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in chickens from small-scale (backyard) commercial poultry farms in Maiduguri.

Materials and Methods: A total of 96 cloacal swab samples were collected. This comprised of 24 samples each from broiler chicks, pullets, layers, and broilers (adults). The samples were examined for the presence of *E. coli* using conventional microbiological culture and biochemical tests. The pure *E. coli* isolates were screened for ESBL production by culturing onto Brilliance™ ESBL agar. Isolates that showed positive reactions with production of bluish or pinkish colonies were tested for susceptibilities against some selected beta-lactam antibiotics which include cefotaxime (30 µg), ceftriaxone (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), and ceftazidime (30 µg). Isolates that exhibited resistance to any two or three of the antibiotics were selected and confirmed by combination disk diffusion method with ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg).

Results: The total occurrence of *E. coli* was 67.6% (65/96) with the highest occurrence of 83.3% (20/24) from broiler chicks and least detection of 54.2% (13/24) from layers. Of this, 32.0% were ESBL-producing *E. coli* with the highest detection rate from layers (38.5%) and least occurrence from pullets (26.7%).

Conclusion: This study revealed the presence of ESBL-producing *E. coli* in chickens from small-scale commercial poultry farms in Maiduguri, thus indicating that chickens may serve as important reservoirs for the transmission of antimicrobial resistant pathogens to humans through the food chain.

Keywords: antimicrobials, chickens, extended-spectrum beta-lactamase, *Escherichia coli*, Maiduguri.

Introduction

The surge in the prevalence of antimicrobial resistance from foodborne pathogens and the natural environment is one of the most significant threats to animals and humans in the 21st century [1-4]. This is due to the narrowing down in the cache of antibiotics both in quantity and quality and due to the gap between antimicrobial resistance and development of antibiotics [5-8]. Food-producing animals are among the most important reservoirs of antimicrobial resistance genes [1,9,10], due partly to the frequent and indiscriminate use of antimicrobial agents as feed additives for prophylaxis and in the treatment of gastrointestinal illness in animals [4,11], thus facilitating the shading of resistance genes and pathogenic foodborne bacteria into the environment [12].

The emergence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in humans and animals has gained global notoriety during the past decade. This comes along with a fiery concern of food animals serving as potential reservoirs of ESBL genes. Since ESBL are plasmid coded, their transfer from animals to humans cannot be overemphasized [13]. In addition, some strains of *E. coli* have been observed to play a pivotal role in the spread and maintenance of ESBL genes [14,15]. ESBL act by inactivating beta-lactam antibiotics such as penicillin and third-generation cephalosporins through hydrolysis of their beta-lactam ring. The enzymes are found majorly in *Enterobacteriaceae*, members of the normal gut flora causing opportunistic to severe bloodstream and urinary tract infections [16-18].

The poultry industry has assumed greater importance in improving employment opportunities and animal food production in Nigeria [19-21]. Earlier reports showed that poultry production accounts for

about 36.5% of sources of protein in Nigeria [22,23]. However, the viability and growth of this sector is hampered by diseases which have consequently resulted in reduced output in terms of production [12]. In many communities in Nigeria, small-scale poultry farms constitute a large portion of poultry farming activities, and previous studies have reported that this poses a major risk in the dissemination of multidrug-resistant pathogens in the environment [24].

In view of the increasing concerns about ESBL-producing *E. coli* in food animals and the possible risk that they pose to public health [25], this study was carried out to investigate the presence of ESBL-producing *E. coli* in small-scale poultry farms within Maiduguri.

Materials and Methods

Ethical approval and informed consent

The study design was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri. Also, informed consent from farmers was obtained for the inclusion of their farms in the study.

Sampling

A total of 96 cloacal swab samples consisting of 24 samples each from broiler chicks, broilers, layers, and pullets were collected and used in this study. The samples were collected from chickens on deep-litter systems in small-scale farms located at Lake Chad, Damboa Road, Mairi, and New GRA wards of Maiduguri. Cloacal swab samples were collected with the aid of pre-labeled sterile swab-sticks dipped in peptone and packed in polystyrene cooler containing ice-packs for immediate transportation to Bacteriology Research Laboratory, Department of Veterinary Microbiology, University of Maiduguri.

Bacterial isolates

The bottles containing cloacal swabs were incubated for 2 h at 37°C aerobically. After enrichment, the samples were then streaked onto freshly prepared eosin methylene blue (EMB; Oxoid, UK) agar and incubated aerobically at 37°C for 24h. *E. coli* colonies on EMB with the appearance of very dark and with a metallic green sheen were sub-cultured onto Nutrient Agar (Oxoid, UK) and incubated at 37°C for 24 h. The colonies were subjected to gram staining for cellular morphology and a series of biochemical tests according to Jang *et al*. (2004). Identified *E. coli* isolates were kept on nutrient agar slant (Oxoid, UK) at room temperature until used.

Antibiotic susceptibility test and confirmation of ESBL production

Pure *E. coli* isolates were screened for ESBL production by culturing onto a Brilliance™ ESBL agar (Oxoid, UK) a chromogenic screening plate for the detection of ESBL-producing organisms. The plates were then incubated at 37°C for 24 h. ESBL-producing *E. coli* were identified by characteristic bluish or pinkish colonies. The ESBL-producing *E. coli* were tested for susceptibility using disk diffusion method as described by Bauer *et al.* [26]. The ESBL-producing *E. coli* were tested against five beta-lactam antibiotics which included cefpodoxime (10 µg), ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). The diameters of inhibition zones were measured and interpreted as per the guidelines of the Clinical and Laboratory Standards Institute [27]. All the isolates that showed resistance to any of the two or three cephalosporins were subjected to confirmation for ESBL production using combination disk diffusion method with ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg) (Oxoid, Basingstoke, UK). The *E. coli* isolates were phenotypically considered as ESBL producers when there is an increase of ≥5 mm in the size of inhibition zone for antimicrobial agents when tested along with clavulanic (ceftazidime-clavulanic acid or cefotaxime-clavulanic acid) in addition to the initial zones of inhibition when ceftazidime and cefotaxime were tested alone

Statistical analysis

The data obtained from this study were summarized and presented for descriptive purposes in the form of tables as percentages. Chi-square analysis was used to test for the level of statistical significance in the association of the occurrence of *E. coli* and ESBL-producing *E. coli* from chickens with respect to chicken types and age using GraphPad Instat3 (GraphPad Software, 2365 Northside Dr. Suite 560 San Diego, CA 92108). Relative risk and confidence interval of 95% were used for the determination of level of statistical significance and the values of $p<0.05$ was considered statistically significant.

Results

Table-1 shows the occurrence of *E. coli* and ESBL-producing *E. coli* in chickens from backyard poultry farms in Maiduguri. The total occurrence of *E. coli* observed was 67.7% (65/96) with the highest occurrence from broiler chicks 83.3% (20/24), followed by broiler 70.8% (17/24) and pullet 62.5% (15/24), while the lowest was from layers having 54.2% (13/24). However, this result showed no statistically significant difference in the occurrence of *E. coli* in chickens based on types and ages ($p>0.05$) (Table-2). In the same vein, the total occurrence of ESBL-producing *E. coli* was 32.3% (21/65) with the highest detection rate from layers 38.5% (5/13) followed by broilers 35.3% (6/17) and broiler chicks 30.0% (6/20), while the least occurrence was from pullets 26.7% (4/15) as shown in Table-1. The result of this study also showed no statistically significant difference in the occurrence of ESBL-producing *E. coli* in chickens based on types and age (Table-3) ($p>0.05$) (Table-3). The presumptive ESBL-producing *E. coli* isolates were tested for susceptibility against

Table-1: Occurrence of *E. coli* and ESBL-producing *E. coli* in chickens from small-scale poultry farms in Maiduguri.

Sample source	Number of sample	Number of positive (%)	
		E. coli	**ESBL-producing *E. coli***
Broiler chicks	24	20 (83.3)	6 (30.0)
Broiler	24	17 (70.8)	6 (35.3)
Pullets	24	15 (62.5)	4 (26.7)
Layers	24	13 (54.2)	5 (38.5)
Total	96	65 (67.7)	21 (32.3)

ESBL=Extended-spectrum beta-lactamase, *E. coli=Escherichia coli*

Table-2: Occurrence of *E. coli* in chickens from small-scale poultry farms in Maiduguri based on chicken types and age.

Factors	Level	Number of sample	Prevalence (%)	p-value	RR	CI
Chicken types	Broiler	24	77.1	REF	NA	NA
	Layers	24	58.3	0.0808	1.321	0.9941-1.756
Age	<4 weeks	24	72.9	REF	NA	NA
	>4 weeks	24	62.5	0.3826	1.167	0.8827-1.542

RR=Relative risk, CI=Confidence interval, *E. coli=Escherichia coli*

Table-3: Occurrence of ESBL-producing *E. coli* in chickens from small-scale poultry farms in Maiduguri based on chicken types and age.

Factors	Level	Number of sample	Prevalence (%)	p-value	RR	CI
Chickens type	Broiler	37	32.4	REF	NA	NA
	Layers	28	32.1	0.9803	1.009	0.4953-2.055
Age	<4 weeks	35	28.6	REF	NA	NA
	>4 weeks	30	33.3	0.6674	0.7792	0.1462-0.4627

RR=Relative Risk, CI=Confidence interval, ESBL=Extended-spectrum beta-lactamase, *E. coli=Escherichia coli*

aztreonam, cefpodoxime (10 μg), ceftazidime, ceftriaxone, and cefuroxime. From the table, highest resistance was recorded against aztreonam (98.5%), followed by ceftriaxone (96.9%), ceftazidime (93.8%), and cefuroxime (75.4%), while the least resistance was from cefpodoxime (67.7%). In the same manner, the highest susceptibility was recorded against cefpodoxime (32.3%) and the least susceptibility was against aztreonam (1.5%) (Table-4).

Table-4: Susceptibility of *E. coli* to beta-lactam antibiotics

Antibiotics	Resistance (%)	Susceptible (%)
ATM (30 μg)	64 (98.5)	1 (1.5)
CPD (10 μg)	44 (67.7)	21 (32.3)
CAZ (30 μg)	61 (93.8)	4 (6.2)
CTX (10 μg)	63 (96.9)	2 (3.1)
CRO (30 μg)	49 (75.4)	16 (24.6)

E. coli=Escherichia coli. ATM=Amoxicillin, CPD=Cefpodoxime, CRO=Ceftriaxone, CAZ=Ceftazidime, CTX=Cefotaxime

Discussion

In this study, a total of 96 cloacal swab samples were collected from chickens in small-scale poultry farms in Maiduguri and screened for the presence of ESBL-producing *E. coli*. Total prevalence of 67.7% of *E. coli* was obtained with the highest occurrence of 83.3% in broiler chicks. This agrees with the findings of Kegode *et al.* [28] where a similar occurrence of *E. coli* from chickens was also reported. The finding of this study also showed that the occurrence of *E. coli* in chickens <4 weeks of age (72.9%) was higher compared to birds of age >4 weeks (Table-2). This finding is not in agreement with other previous studies where the authors reported 44% prevalence of *E. coli* in chicks [29]. The high occurrence of *E. coli* in chicks from this study could be linked to a lack of good sanitary conditions observed in the farm environments during this work. It was noted from this study that most small-scale farmers entrust their farm management to individuals who have little or no formal education and experience in poultry farm practice and therefore give little attention to the hygiene of birds and the environments. Hence, creating a conducive atmosphere for bacterial growth and colonization. In addition, the high occurrence could also be attributed to sampling source and type of samples, and for the fact that *E. coli* is a normal gut flora, [30-32].

In this study, a total occurrence of 32.3% of ESBL-producing *E. coli* was observed in chickens. Similar prevalence of ESBL-producing *E. coli* in chickens was also reported in Zambia by Chishimba *et al*. [6]. This finding is lower than the findings of previous studies [33-35], where higher occurrences of ESBL-producing *E. coli* were reported in chicken meat. It was also observed from this study that the highest occurrence of ESBL-producing *E. coli* was from laying birds (layers) (38.5%). Based on this, it is important to note that layers are normally kept for a longer period and therefore may have prolonged exposure to antibiotics for prophylaxis which might result in the selection of drug-resistant bacterial

pathogens. However, since no statistically significant difference was observed ($p>0.05$) in the occurrence of the pathogen in chickens with respect to chicken-types, it implied that both broilers and layers are at risk of harboring the organism when raised under conditions that support the selection of antimicrobial resistant pathogens. The occurrence of ESBL-producing *E. coli* in broilers is higher than the findings of Shoaib *et al*. [32] where 7.76% occurrence rate was reported. Furthermore, results of analysis of the occurrence of ESBL-producing *E. coli* in chickens based on age showed no statistically significant difference on the occurrence of the organism ($p>0.05$) which implies that both age groups may be at risk of harboring the pathogens if managed under conditions that promote transmission and acquisition of the organism.

According to our results, *E. coli* isolates were resistant to aztreonam (98.5%), ceftriaxone (96.9%), ceftazidime (93.8%), cefuroxime (75.4%), and cefpodoxime (67.7%). These findings are in contrast to that of Gundogan and Avci [9] where lower resistance to cefotaxime (33.3%), ceftazidime (8.9%), and ceftriaxone (8.9%) was reported. The high antibiotic resistance among *E. coli* recorded in this present study might be due to uncontrolled administration of antibiotics to chickens.

Conclusion

The study affirmed the presence of ESBL-producing *E. coli* in chickens from backyard poultry farms in the study area. These isolates also showed a high level of antibiotic resistance to third-generation cephalosporins and the monobactam, aztreonam. This is of serious public health significance since birds are reared in close proximity to human population and may disseminate these resistant pathogens in the environment and in-contact farm personnel.

Authors' Contributions

IDK, JAM, and AAO conceived and designed the work and put the first draft of the manuscript. ND, DLM, and KUE participated in some field work and data collection. AAB and IDK analyzed the data obtained from the study and contributed to the final draft of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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