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Prevalence of Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* in Broiler Chickens in Yaoundé Capital City of Cameroon

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Abstract

Background: *Escherichia coli* are ubiquitous bacteria colonising both humans and animals. Extended spectrum *β*-lactamase-producing *E. coli* has been selected as a suitable indicator for the monitoring and surveillance of antimicrobial resistance. Death due to resistant bacteria is continuously rising in Cameroon, but the contribution of the aviary sector is not well studied. Therefore, this study aimed to investigate the resistance profile of extended spectrum beta-lactamases-producing *Escherichia coli* strains, isolated from faeces of broiler chickens in Yaoundé, capital city of Cameroon. **Methods:** A cross-sectional descriptive study was carried out from February to June 2020. *Escherichia coli* were isolated from samples of broilers in poultry farms in Yaoundé and submitted to the extended spectrum *β*-lactamase screening. The logistic regression was used to assess the statistical association of a significance threshold p-value of 0.05. **Results:** Out of 385 faecal samples collected in broiler farms, 114 *Escherichia coli* isolates were obtained out of which 30 (26.32%) were Extended Spectrum Beta-Lactamases-producing *Escherichia coli*. These isolates revealed high resistance to all antibiotic families. Poor storage conditions for feeds and the proximity to latrines, the troughs on the ground, the lack of foot bath and uniforms, the inadequate treatment of faec-

es, the poor usage of preventive antibiotics and the lack of water treatment have been identified as risk factors to faecal carriage of ESBL-producing *Escherichia coli*. **Conclusion:** This work reveals the emergence of Extended Spectrum Beta-Lactamases-producing *Escherichia coli* in poultry farms in Yaoundé and the failure in the biosecurity system. As such, the awareness of poultry breeders on the respect of biosecurity measures may be an effective tool to tackle antimicrobial resistance, specifically in livestock industries using a One Health approach.

Keywords

Extended Spectrum Beta-Lactamase, *Escherichia coli*, Antibiotic Resistance, Broiler Chicken, Yaoundé, Cameroon

1. Introduction

Livestock production plays a fundamental role in food security, nutrition, biodiversity conservation and improving the income of urban and rural households. In Cameroon, with an estimate of 52 million headcounts (broiler and layers), poultry production provides at least 34.26% of the total meat harvested from the terrestrial food-producing animals per year [1]. In this country, most poultry farms have no waste or litter treatment facilities, and poultry waste is often used as organic fertilizer or as feed supplements, especially in fish ponds. This may increase the risk of exposure of antimicrobial resistant bacteria from waste to humans, as the waste might contain zoonotic bacteria including pathogenic *Escherichia coli*. The antimicrobial agents are used as growth promoters in poultry feeds to increase poultry production [2]; unfortunately, most of these antimicrobials are not fully absorbed in the chicken gut and up to 90% of the administered dose is excreted in the faeces [3] [4] [5] [6] [7].

Escherichia coli, a mutualistic bacterium found in humans and animals, constitutes approximately 99% of the aerobic bacterial intestinal flora. This bacterium has the ability to evade various antimicrobial agents due to its capacity to develop numerous resistance mechanisms including the production of extended-spectrum β-lactamases (E-ESBL). The practice of using antimicrobials in animal feeds may alter the gut flora by creating selective pressure for resistant bacterial populations, such as resistant *E. coli* that could end up in the environment and food chains [4] [8]-[15]. Therefore, continued monitoring and surveillance of resistant pathogens across the human-animal environmental interface using *Escherichia coli* as indicator, represents a cornerstone for decision making and mitigation strategies.

Epidemiological data reveal a worrying situation of the spread of these antimicrobial resistances within poultry farms. Previous studies reported the prevalence of ESBL producing *Escherichia coli* strains in sub-Saharan African region, with 19.23% in Chad [14], 11.2% in Senegal [5] and 4.1% in Nigeria [6]. In Ca-

meroon, a number of studies [10] [16] [17] have assessed poultry litter contaminations by *Escherichia coli* resistant to critically important antimicrobials for human and animal use and the implications for the public health [11], emphasizing the need for surveillance in the livestock production for food security. Lack of surveillance data in one aspect or another might increase delays in detection or response to newly emerged threats to public health. Therefore, the present study was initiated with the global aim to characterize the profile of the resistant Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* strains isolated from broiler faeces in Yaoundé, the Capital city of Cameroon.

2. Material and Method

2.1. Study Site and Design

This cross-sectional study was carried out from February to June 2020 in Yaoundé the Capital city of Cameroon. The samples collected from the poultry farms were analyzed in the National Veterinarian Laboratory (LANAVET), Yaoundé. The size of the sample was calculated using the Lorentz formula. The number of broilers randomly sampled per farm was function of the farm size (number of subjects in the farm) using the proportionality principle. A cluster sampling at many degrees was realized through random and successive selection of quarters and farms. The minimum sample size was calculated using the Lorentz formula as follows:

$$N = P\frac{(1-P)z^2}{d^2} = 0.5\frac{(1-0.5)1.96^2}{0.05^2} = 385$$

The farms possessing broiler flocks aged 0 - 45 days were included in the study, while broilers aged 0 - 45 days that were sick and died were excluded. Prior to the sample collection, a designed questionnaire validated against a pilot study realized in the West region of Cameroon was issued to the breeders to gather possible risk factors. The question sample included in the questionnaire was as follows: 1) What is your main purpose when administering the antibiotics? 2) Who supplied you with antibiotics? 3) Who gave you advises on the choice for antibiotics? 4) What antibiotic do you take when you are ill? 5) Where do you dispose the feed, in the floor? 6) Do you possess catchment water and ground troughs? 7) Is there a latrine in the surroundings? 8) Do you possess footbaths? 9) Do you possess uniforms for breeding? 10) Do you treat the poultry water? 11) Describe the water treatment process.

2.2. Sample Collection and Laboratory Analysis

Cloacal secretions were obtained using sterile swabs. The labelled samples were coded and conveyed in coolers containing icepacks. The culture media were prepared and the samples were viewed under the microscope followed by the plating in 90 mm petri dishes containing Methylene Blue Eosin Agar (MBE) with a Ceftiofur solution of 1 mg/L to obtain only Ceftiofur resistant strains, and were then incubated at 37˚C for 18 to 24 hours. A control test was carried out to

ensure the absence of contamination, and was followed by a fertility test using the reference strain *Escherichia coli* (ATCC25922) to attain fertile media. *Escherichia coli* strains were identified using the API 20 E Gallery (Biomerieux), whereby each gallery was inoculated with a bacterial suspension previously prepared with the 0.5 McFarland standards. Testing of *Escherichia coli* strains for an antibiotic susceptibility was performed using the Kirby-Bauer method and Muller Hinton agar diffusion as recommended by AC-FSM 2019. Table 1 below shows antibiotics that were tested according to the AC-FSM 2018 Veterinary recommendations. ESBL screening was done according to the double disk synergy testing, whose production was detected by the appearance of a Champaign cork image materialised between the clavulanate (amoxicillin + clavulanic acid) disk and a C3G or Aztreonam disks. Inhibition diameters were measured with a vernier calliper to assess susceptibility profile.

Table 1. Antibiotics used in the study.

Antimicrobial	Family	charge (µg)
Amoxicilline/clavulanic acid	Oxapenam	20 - 10
Amoxicilline	Amino-penicillin	25
Ceftazidime	C3G	10
Cefoxitine	C2G	30
Cefepime	C3G	30
Cefotaxime	C3G	5
Ceftiofur	C3G	30
Ceftriaxone	C3G	30
Ticarcilline	Carboxy-penicillin	75
Piperacillin	Acyl-amino-penicillin	30
Imipenem	Carbapenem	10
Ertapenem	Carbapenem	10
Aztreonam	Monbactam	30
Ciprofloxacine	Fluro-quinolones	5
Ofloxacine	Quinolone	5
Acide nalidixique	Quinolone	30
Kanamycine	Aminoside	30
Gentamicine	Aminoside	500
Amikacine	Aminoside	30
Tetracycline	Cycline	30
Fosfomycine	Fosfomycine	200
Cotrimoxazole	Diaminopyri-midine	1.25 - 23.75

2.3. Data Analysis

Data collected were entered into excel for management and statistical analysis using Epi-Info 7 software. The confidence interval was calculated when necessary. Logistic regression permitted to find the predictive factors of avian digestive carriage of ESBL producing *E. coli* considering a significance threshold p-value of 0.05.

2.4. Ethical Consideration

The ethical clearance was obtained from the institutional committee of Université des Montagnes (Ref: N02020/117/UdM/PR/CIE). The National Veterinary Laboratory—LANAVET, the Regional Delegate of the Ministry of Livestock, Fisheries and Animal Industries—MINEPIA and the Divisional Delegate of MINEPIA all issued the ethical clearance with Reference numbers 066/19/LANAVET-AYDE/D, N0000161/L/MINEPIA/SG/DREPIA-CE/SRDPIA and N049/L/MINEPIA/DREPIAC/DDEPIA-MFDI, respectively. The breeders' informed consents were obtained.

3. Results

3.1. Determination of Resistance Prevalence of *Escherichia coli* Strains Isolated from Faecal Carriage in Broilers to *β*-Lactamins

A total of 385 chicken faeces samples were collected out of which 114 *Escherichia coli* isolates were obtained. The frequency distribution of Extended Spectrum Beta-Lactamases-producing *Escherichia coli* is presented in Figure 1.

It reveals that farm 2 and 6 were mostly contaminated with Extended Spectrum Beta-Lactamases-producing *Escherichia coli* (45.45% and 42.86%, respectively) as compare to others.

3.2. Prevalence of ESBL Producing *Escherichia coli* Isolates Resistant to *β*-Lactams and Co-Resistances to Other Antibiotics Families

Extended Spectrum Beta-Lactamases-producing *Escherichia coli* exhibited high resistance to *β*-lactams and other antibiotic families (Figure 2 and Figure 3).

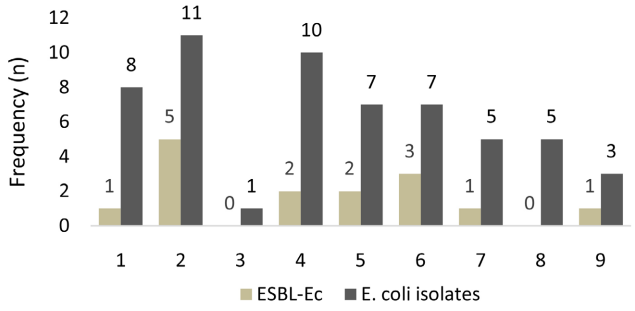

Figure 1. Frequency distribution of ESBL producing Escherichia coli isolates according to poultry farms.

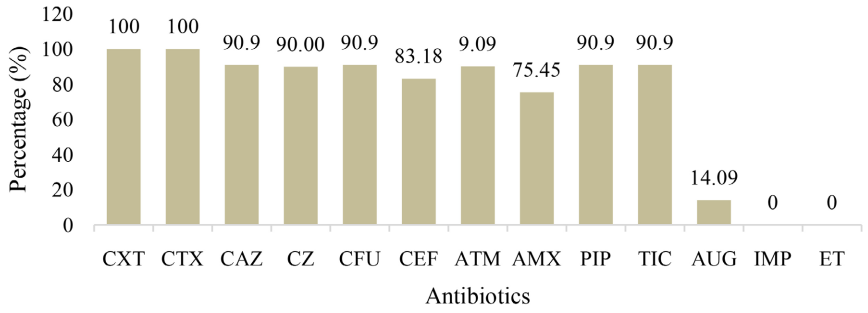

Figure 2. Prevalence of ESBL producing *Escherichia coli* isolates resistant to *β*-lactams. CXT: Cefoxitine; CTX: Cefotaxime; CAZ: Ceftazidime; CZ: Cefazoline; CFU: Ceftiofur; CEF: Cefepime; ATM: Aztreonam; AMX: Amoxicilline; PIP: Piperacilline; TIC: Ticarcilline; AUG: Amoxicilline + Clavulanic Acid, IMP: imipenem, ET: Ertapenem.

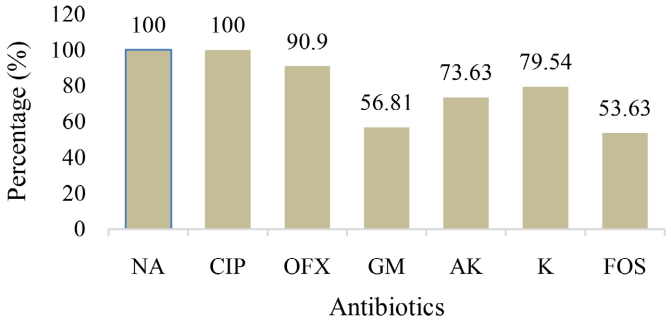

Figure 3. Prevalence of *Escherichia coli* isolates resistant to other families of antibiotics. NA: Nalixidic Acid; CIP: Ciprofloxacine; OFX: Ofloxacine; GM: Gentamycine; AK: Amykacine; K: Kanamycine; FOS: Fosfomycine; COT: Cotrimoxazole; TET: Tetracycline.

A resistance prevalence of 100% was observed toward Cefoxitin, Cefotaxim, Nalidixic acid and Ciprofloxacine. Oppositely, null resistance was observed toward carbapenems. High resistance prevalence was also observed toward other antibiotic disks except for clavulanate.

3.3. Analysis of Predictive Factors to Faecal Carriage of ESBL-Producing *Escherichia coli* Strains

The univariate analysis (Table 2) shows that poor storage conditions for feed and feeders (on the ground), proximity to latrines, lack of a footbath, lack of a uniform, lack of maintenance of fecal droppings, catchment water, floor troughs, poor use of antibiotics as a preventive measure, lack of water treatment and age range of broilers from 10 - 22 days significantly influence the contamination of broiler faeces by *Escherichia coli* ESBL strains (p-value < 0.05).

The multivariate logistic regression (Table 3) confirms that poor storage conditions for the feeder (on the ground), proximity to latrines the absence of a footbath and uniform, the lack of maintenance of fecal droppings, the water and water troughs on the ground, the heavy use of antibiotics as a preventive measure and the lack of water treatment of broilers are risk factors to fecal carriage of ESBL-producing *Escherichia coli* isolates (p-value < 0.05).

Table 2. Simple logistic regression to search for predictive factors to faecal carriage of ESBL producing *Escherichia coli*.

Variables	No/Yes	Total Headcount (N)	Presence of *E. coli* ESBL		p-value
			N	(%)	
Poultry training	No	138	18	13.04	Ref
	Yes	62	7	11.29	0.04
Poor storage conditions of feed and feeders (on the ground)	No	53	3	5.66	Ref
	Yes	147	30	6.8	**0.001**
Proximity to latrines	No	68	5	7.35	Ref
	Yes	132	18	13.63	**0.002**
Lack of a foot bath and uniform	No	44	7	15.9	Ref
	Yes	156	12	7.7	**0.01**
Lack of maintenance of faeces	No	53	3	12	Ref
	Yes	147	30	11.3	**0.02**
Catchment water and ground troughs	No	55	9	16.36	Ref
	Yes	145	17	11.72	**0.02**
Misuse of antibiotics (Preventive)	No	66	2	3.03	Ref
	Yes	134	7	5.23	**0.01**
Lack of water treatment	No	27	7	25.92	Ref
	Yes	173	19	10.98	**0.003**
Fruit trees	No	169	33	19.52	Ref
	Yes	31	17	54.83	0.4
Age range	[10 - 22 days]	58	20	34.48	Ref
	[40 - 42 days]	58	7	12.06	0.1
	[43 - 44 days]	84	10	11.9	0.2
Presence of rodents	163		19	11.65	Ref
	37		8	21.62	
Presence of domestic animals	No	171	17	20.68	Ref
	Yes	29	6	9.94	

Table 3. Multiple logistic regressions to search for predictive factors to faecal carriage of ESBL producing Escherichia coli.

Variables		OR	CI at 95%	p-value
Poor storage conditions storage of feed and feeders (on the ground)	**No**	Ref.	[2.23; 14.03]	**0.02**
	Yes	7.79		
Proximity to latrines	**No**	Ref.	[1.9; 9.05]	**0.42**
	Yes	1.89		

Continued

Lack of footbath and uniforms	**No**	Ref.	[1.4; 4.5]	**0.01**
	Yes	1.89		
Lack of maintenance of faeces	**No**	Ref	[3.98; 30.5]	**0.04**
	Yes	0.36		
Catchment waters and ground troughs	**No**	Ref	[1.04; 12.15]	**0.03**
	Yes	0.48		
Misuse of antibiotics (Preventive)	**No**	Ref	[2.32; 11.19]	**0.04**
	Yes	0.22		
Lack of water treatment	**No**	Ref	[1.94; 14.18]	**0.02**
	Yes	0.44		

OR: Odd Ratio; CI: Confidence Interval.

4. Discussion

The objective of this study was to contribute to the control of resistance phenotypes of ESBL (extended spectrum beta-lactamase) producing *Escherichia coli* strains isolated from broiler faeces in poultry farms in Yaoundé, capital city of Cameroon.

With regard to antibiotic use, and according to Simo Louokdom *et al.* [8], it is suggested that the impact on livestock would be greater than that attributed to the misuse of drugs in human medicine. 26.31% *E. coli* isolates produced ESBL in our study is high compared to the study conducted by Vounba *et al.* [6] that showed a proportion of 19.8% and 11.2% in Vietnam and in Senegal, respectively. This highlights the ingenuity of *Escherichia coli* strains that multiply and increase in resilience in the digestive tract while escaping Beta-lactams mechanisms of action. Consequently, ESBL producing *Escherichia coli* confers to bacteria a remarkable resistance to beta-lactams and a co-resistance to other families of antibiotics [18]. These findings are in accordance with the work of Djuikoue *et al.* [18] who found intestinal carriage of ESBL producing *E. coli* in women with urinary tract infections, thereby strengthening the evidence of other existing activities of ESBL *E. coli* other than just inhibitory to beta-lactams.

The readiness with which bacteria share the gene encoding for ESBL through mobile genetic elements such as the plasmids, the transposons or the integrons shows that this genetic elements constitute an alarm. Henceforth, *Escherichia coli* is characterised as a multi-resistant bacterium, which may be due to its commensal nature, hence permanently in contact with consumed antibiotics. A number of studies [10] [11] [12] [13] have indicated that the administration of antibiotics at sub-lethal doses for disease prevention could be responsible for the amplification of resistances. To this end, we can suggest that the frequent and abusive use of antibiotics constitutes a source of resistance selection, therapeutic failure, a decrease in zootechnical performance and consequently impinges on

Public Health.

For the use of antimicrobials during disinfection by farmers after the passage of a flock, good zoo-hygienic practices may be attributed to their level of education. As observed in our work, most farmers (60%) have university education; this tendency is similar with the work of Yawat *et al.* [9] that showed 75% of farmers having completed university studies. So, a high education level predisposes farmers to better understanding the reasons for disinfection using water and antiseptics.

The dissemination of antibiotic residues in the environment could be as a result of the droppings used as manure after the release of a band or fertiliser for agricultural use, which may therefore transmit resistance genes to bacteria present in nature to plants and even to aquatic environment. This process is realistic due the affordable fertiliser and readily available on local markets for farmers and breeders. In addition, the poor condition of feeds and troughs (on the ground) storage, the proximity to latrines, the absence of a footbath and uniform, the lack of faeces maintenance, the water catchments and troughs on the ground, the poor use of antibiotics as a preventive measure, the absence of water treatment and the age range (10 - 22 days) in different poultry farms visited during our study represent associated factors to faecal carriage of ESBL-producing *Escherichia coli* strains.

The risk factors identified to trigger faecal carriage of ESBL-producing *Escherichia coli* by broiler was poor condition of storage for the feeder (on the ground), proximity to latrines the absence of a footbath and uniform, the lack of maintenance of faecal droppings, the water and water troughs on the ground, the heavy use of antibiotics as a preventive measure and the lack of water treatment.

The limitations of this study were the lack of genotypic assays, and the use of phenotypical assays for ESBL detection, which is relatively less specific and less sensitive.

5. Conclusion

At the end of this study, which focused on the characterization of the resistance phenotypes of *Escherichia coli* strains isolated from the faeces of broilers in the poultry farms in Yaoundé capital city of Cameroon, prevalent ESBL producing *Escherichia coli* showed high resistance and co-resistance. However, this multi-resistance to antibiotics makes it difficult to manage the subjects in terms of both prevention and therapy of infectious diseases. These levels or the presence of resistance phenotypes are elements that risk unbalancing the balance advocated by ONE HEALTH approach, whose aim is to put in place biosafety measures to maintain the sanitary balance at animals, humans or environmental levels. This study is evidence that the emergence of ESBL-producing *Escherichia coli* is principally due to the abusive use of antibiotics and that contamination of broiler by these multi-drug resistant strains is mainly caused poor hygiene practices.

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Consent for Publication

All authors consented for publication.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

Authors declare no competing interests.

Authors' Contributions

CID conceived the project, designed the study, searched for relevant literature, scrutinized all relevant information and wrote the first draft of the manuscript. CID and CNT conducted and coordinated the field study. CNT, MFo, MFr collected and processed the samples and data. CID, CSN, ATD analyzed the data. AW supervised the laboratory work. VTN revised and finalised the manuscript. All authors read and approved the final manuscript.

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