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Antimicrobial Resistance Pattern of *Escherichia coli* Strains Isolated from Meat and Fish Products Collected in Retail Market in Douala, Cameroon

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Abstract: Foodborne infections bacterially mediated increase morbidity and mortality rates, especially in developing countries where acute hygiene and sanitation are wanting. Associated with antimicrobial resistance, these infections are foremost among key public health concerns. In this study, we assessed the phenotypic characterization of pathogenic *Escherichia coli* strains contaminating fish and meat products sold and destinated for human consumption in Douala. As such, 95 samples of fish and meat products were randomly collected from 8 markets and 11 supermarkets of Douala urban council. *E. coli* was isolated and identified by culture-dependent and biochemical techniques and antimicrobial susceptibility test was assessed using the Kirby Bauer method. The results showed that 89.5% of fish and meat products were contaminated by ß-glucuronidase positive *E. coli* and 42.1% by Extended Spectrum β-lactamase *E. coli*. Its resistance profile showed extremely high resistant isolates to β-lactam antibiotics (90.9%-100%), very high to high resistance for third generation cephalosporin (43.6%-100%), high to moderates resistance (15.4% - 40%) for macrolides antibiotics families, moderate resistance to imipenem (10% à 18.2%) and multiresistance in 94. 9% of isolates. Additionally, our data revealed that fish and meat samples were potential sources of dissemination of antimicrobial resistant *E. coli* on food chain in Douala. This constitutes a serious health risk problem for human population and highlights the need for one health approach to better understand and manage this issue in Cameroon.

Keywords: *Escherichia coli, β-lactamase E. coli, phenotypic characterization, multidrug resistant, retail market, Douala*

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1. Introduction

Enteric infections are a major threat to public health in developing countries [1]. Their occurrence is strongly associated with inadequate drinking water supplies and poor food hygiene [1]. *Escherichia coli* is one of the major hazards in food and water sources [2-3], thus indicating poor sanitation and a potential risk to food safety. *E. coli* is considered as the most important pathogenic foodborne bacterium and a leading indicator of faecal contamination [4]. Some of its strains also known as main commensal bacteria in the human gut have acquired specific virulence factors and antimicrobial resistance genes (ARGs) [5], complicating the treatment of infections. Furthermore, the emergence of antibiotic resistance in foodborne pathogens presents additional complications [6]. According to the WHO, antimicrobial resistance (AMR) is one of the serious health problems of our time [7] and it requires a multisectorial strategy such as "One Health" initiative [8]. A global study of drug-resistant bacteria identified *E. coli* as one of the leading pathogens responsible for 929,000 AMR-related deaths [7]. Due to its strong ability to acquire AMR genes through horizontal gene transfer and act as both donor and recipient when transferring ARGs, *E. coli* has been used as a biomarker to detect AMR in various medical or environmental settings, including food, feed processing animals, hospitals, and the human-wildlife interface [9,10]. The increase level of resistant *E. coli* strains in developing countries and the prevalence of antimicrobial resistance genes in poultry and meat products has been associated with the inappropriate use of antibiotics by small poultry farmers [11,12]. This phenomenon is an ultimate global health threat and the situation is getting worst with the emergence of multiple

drug resistance bacteria (MDR) in animal foods [13]. In developing countries, AMRs are overburdened due to the use of antibiotics as growth promoters by farmers, feed traders, drug traffickers, and the lack of approved legislation by relevant government agencies [14]. Multidrug-resistant (MDR) bacteria are increasing in every corner of the developing world, increasing treatment costs [15]. In 2020, a review published by Kumar et *al.* indicated that chicken meat was one of the potential causes of Multi-Drug Resistant (MDR) *E. coli* infection [16]. In addition, WHO report on country situation analysis of AMR based on a two years surveillance with the national sanitary authorities showed a lack of national AMR policies, lack of sensitization programs, and lack of data on AMR surveillance and inappropriate use of antimicrobials in sub-Saharan African countries [17]. Furthermore, no country in Africa, including Cameroon, has a national surveillance system that regularly generates representative and reliable data on antibiotic use and resistance [18]. In Cameroon over the past decades, an increasing prevalence of drug-resistant bacteria isolated during human infections was recorded [19]. Few studies have been carried out on drug resistance bacteria isolated from cloacal, carcasses and eggs samples [20-22]. To the best of our knowledge, reports on how food samples assigned for human consumption could contribute to spreading antimicrobial resistant bacteria along the food chain are wanting. A recent review and meta-analysis on antimicrobial resistance from one health approach revealed from the 66 studies pooled, 45 reported antibiotic resistance in humans, 9 reported consequences of antibiotic resistance in animals, and 12 reported antimicrobial resistance in environment [23]. However, there were no reports on the antibiotic resistance in food products. According to Food and Agriculture Organization (FAO), poultry production in Cameroon represented at least 34.26% of the total meat harvested from the terrestrial food-producing animals in 2020 with an estimated headcount of 52 million broilers [24]. The city of Douala, a cosmopolitan city in Cameroon, is considered as the leading center of animal protein production in Cameroon and one of the largest consumers of beef, poultry and pork in the country. Thus, the impact of the presence of pathogens zoonotic bacteria in animals and food is a national public health concern. Notably, antimicrobial resistant strains from animal hosts can transmit a variety of diseases to humans through food and water intake. This study which is part of a larger research project aimed to provide preliminary data on the prevalence, and AMR profile of *E. coli* isolated from meat and fish products in Douala, Cameroon.

2. Materials and Methods

2.1. Study Design and Site

A cross-sectional study was conducted in Douala urban council in the Littoral region of Cameroon from October 2021 to July 2022. A preliminary investigation was carried out to identify the different retails markets and sale points of raw chicken, beef, pork and fish samples.

2.2. Sample Collection

Ninety-five raw meat (beef, chicken, pork) and fish samples were randomly collected from 8 retails markets and 11 supermarkets in Douala city (Figure 1). Different retails markets were chosen based on their ability to sell different patterns. Sampling was done according to ISO 13307:2013 and ISO/TS 17728:2015 standards recommendations [25,26]. and sample size was estimated according to Schwartz formula with the following parameters, Z(1-a/2) as standard normal variable (1.96) at 5 % Type I error ($p<0.05$), assuming a prevalence of *E. coli* contamination of 30%, with type I error ($p<0.05$) and 95% confidence level. Sample type was selected according to Caprioli et *al.* [27] and samples were collected using individual zipper sterile plastic bag and transported to the laboratory in refrigerated boxes.

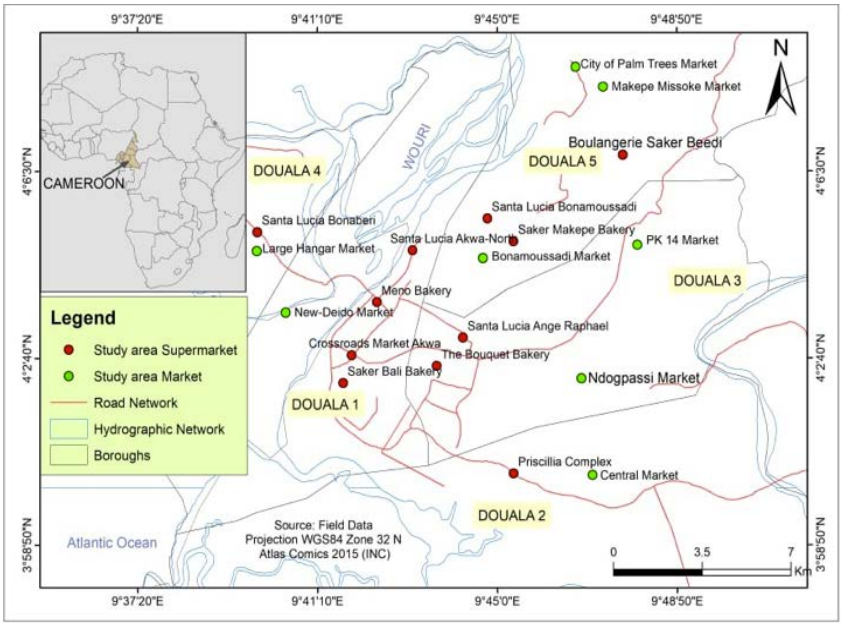

Figure 1. Cartography of sampling site in Douala

2.3. Microbiological Analyses

2.3.1. Samples Preparation

Following the instructions of ISO 6887-1: 2017, 25 g of each sample was added to 225 ml of sterile Buffered Peptone Water (BPW Biokar, France) and homogenized during 1 min using a peristaltic homogenizer (LB 400 VWR) to obtain a 1/10 dilution [28]. The suspension obtained was resuspended in BPW broth and incubated for 24H at 37°C.

2.3.2. Detection of β-glucuronidase Positive *E. coli* (*BGP_EC*)

The detection of β-glucuronidase positive *E. coli* was carried out following the method described in ISO 16649-2: 2001. Briefly, 1 ml of each pre-enrichment suspension was placed in a Petri dish to which 20 ml of sterile Tryptone Bile X Glucuronide Agar (TBX, Biokar, France) was added. Plates were homogenized, left at room temperature to gel and incubated aerobically at 44°C for 24 hours [29]. *BGP_EC* colonies that appeared blue or blue-green were subcultured onto Tryptone Soy Agar (TSA, Biokar, France) and incubated at 37°C for biochemical identification according to the API 20 E identification system (Bioméreux, France). Biochemical reactions were readed according to the readout table of the technical data sheet and identified by reference to the Analytical Profile Index. *BGP_EC* isolates confirmed as *E. coli* were stored at -20 °C for further analysis.

2.3.3. Detection of Extended Spectrum β-lactamase_*E. coli (ESBL_EC)*

2.3.3.1. Isolation of Presumptive Extended Spectrum β-Lactamase_*E. coli*

For the isolation of presumptive ESBL_EC, Tryptone Bile X Glucuronide Agar (TBX, Biokar, France) was supplemented with Cefotaxime 4 mg/L. Then a full inoculation loop (10µL) of each pre-enriched suspension was inoculated onto a Petri dish containing TBX + cefotaxime and incubated aerobically at 44 °C for 24 h. *ESBL_EC* colonies appearing blue or blue-green were subcultured on Tryptone Soy Agar (TSA, Biokar, France) and incubated at 37°C for biochemical identification assays according to the API 20 E identification system (Bioméreux, France). Biochemical reactions were interpreted according to the technical data sheet table and identification was done by reference to the Analytical Profile Index. Presumptive *ESBL_EC* isolates confirmed as *E. coli* were subcultured on Tryptone Soy Agar (TSA, Biokar, France), incubated at 37°C for 24 h, and stored at -20°C for further analysis.

2.3.3.2. Confirmation of Presumptive Extended Spectrum β-lactamase_*E. Coli* isolates

Confirmation of ESBL *E. coli* isolates was assessed for all presumptive *ESBL_EC* biotype isolates following double disk synergy test between Amoxicillin-Clavulanic acid 20/10 µg disk and Cefotaxim 5 µg disk. Presumptive ESBL *E. coli* isolates were confirmed by expansion of third generation cephalosporin (Cefotaxim 5 µg) inhibition zone towards Amoxicillin-Clavulanic acid disk. ESBL production was appreciated through the appearance of a Champaign cork image (Figure 2) between the clavulanate (Amoxicillin + clavulanic acid) disk and Cefotaxim or Aztreonam disks.

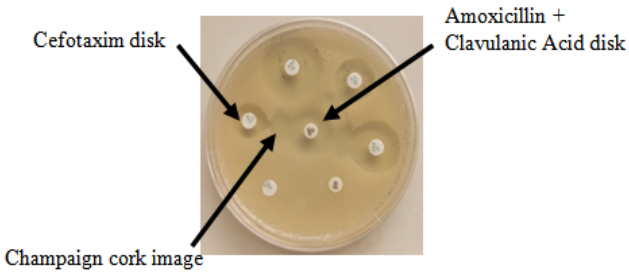

Figure 2. Double disk synergy test plate presenting a Champaign cork image

2.4. Antimicrobial Susceptibility Testing

Table 1. Concentrations and interpretation breakpoints of the various antimicrobial agents used in this study [30]

Antimicrobial agent (Code)	Disc drug concentration (µg)	Breakpoints (mm)		
		Sucepti ble (S)	Intermed iate (I)	Resista nt (R)
Amoxicillin (AMX)	20	≥19	/	<19
Amoxicillin - Clavulanic Acid (AMC)	20/10	≥19	/	<19
Ticarcilin (TIC)	75	≥23	20-23	<20
Cefotaxim (CTX)	5	≥20	17-20	<17
Ceftriaxon (CRO)	30	≥25	22-25	<22
Aztreonam (AZM)	30	≥26	21-26	<21
Azithromycin (ATM)	15	≥ 17	/	<17
Imipenem (IMP)	10	≥ 22	19-22	<19
Chloramphenicol (CHL)	30	≥ 17	/	<17
Amikacin (AMK)	30	≥ 18	/	<18
Ofloxacin (OFX)	5	≥ 24	24-22	<22
Tobramycin (TOB)	10	≥16	/	<16
Nalidixic Acid (NAL)	30	≥ 14	/	<14
Ciprofloxacin (CIP)	5	≥25	22-25	<22

Antimicrobial susceptibility testing was carried out according to the Kirby Bauer disk diffusion method. For sample preparation, a total of 79 *E. coli* isolates stored at -20°C were randomly selected, subcultured and purified on a non-selective agar (TSA, Biokar, France). Then, 3-5 colonies having the same morphology were selected from TSA, transferred into tubes containing 4-5 ml of peptone water broth (Biokar, France) and incubated at 35–37∘C during 2–8 hrs. The turbidity of the preincubated broth and the suspension of bacteria were then adjusted by comparison with 0.5 McFarland using a densitometer (Densimat, Den-1B Biosan). The bacterial suspension was inoculated on Mueller Hinton agar (Biokar, France) with a sterile swab to cover the whole surface of the plate. A total of 14 antibiotics belonging to 5 families, namely β-lactam (Amoxicillin 20µg, amoxicillin-clavulanic acid 20/10 µg, Ticarcilline 75 µg, Cefotaxim 5 µg, Ceftriaxone 30 µg, Aztreonam 30 µg and Imipenem 10 µg), phenicols (Chloramphenicol 30 µg), aminoglycosides (Tobramycin 10 µg, Amikacin 30 µg), Macrolides (Azithromycin 15 µg) and quinolone (Ofloxacin 5 µg, Nalidixic acid 30 µg,

Ciprofloxacin 5 µg) were tested and the results were interpreted according to European Committee on Antimicrobial Susceptibility (EUCAST 2021) (Table 1) recommendation breakpoint for each antibiotic. *Escherichia coli* ATCC 25922 was included as negative control.

2.5. Data Analysis

Variable data obtained were first treated using an Excel sheet before being analyzed with IBM SPSS Statistics Version 25 software. A Chi-2 test of independence was performed to analyze the association between *E coli* contamination and the different meat and fish samples at a significance level of 5%. The resistance rate per 100 for each antimicrobial was calculated as the percentage of isolates showing resistance to a specific antimicrobial with the following formula.

Resistance rate (%) = [(number of resistant isolate) / (number of isolates tested)] X 100

According to Papadopoulos et *al.* [31], resistance rates were classified as extremely high (% rate > 70%), very high (% rate: > 50 to 70), high (% rate > 20 to 50), moderate (% rate > 10 to 20), low (% rate > 1 to 10), very low (% rate 0.1 to 1), and rare (% rate < 0.1). The significance of phenotypic resistance rates in *E. coli* isolates and the frequencies of antimicrobials resistant isolates in different sample types were assessed using chi-square analysis. *E. coli* isolates which were resistant to more than three classes of antimicrobials were considered as multidrug-resistant.

3. Results

3.1. Sample Distribution

A total of 95 samples were collected from different markets and sales points of Douala Urban council. The different samples were distributed as presented in Figure 3.

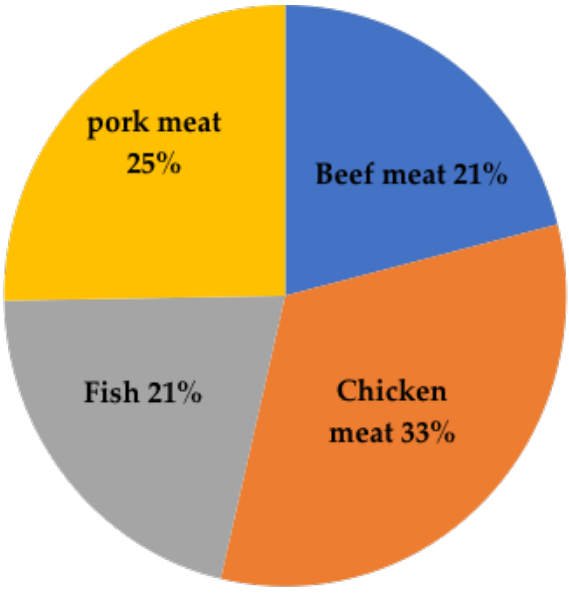

Figure 3. Distribution of meat and fish samples collected for the study

3.2. Frequencies and Distribution of *E. coli* in Food Samples.

3.2.1. Contamination Frequencies of β-glucuronidase Positive *E. coli* (*BGP_EC*)

From 95 samples collected, 85 (89. 5%) were contaminated by *BGP_EC*, with predominant rate of 100 % (20/20) from beef, 95.8 % (23/24) from pork meat, 83.9% (26/31) from chicken meat and 80.0% (16/20) from Fish samples (Table 2). Khi-2 test results revealed that there was no significant difference of β-glucuronidase positive *E. coli* contamination among the different samples (khi-2 of Pearson: 6.32; p=0.09>0,04).

Table 2. Contamination Frequencies of β-glucuronidase positive *E. coli* in collected samples

Sample type	Number of samples tested	Number of positive samples	Frequencies (%)
Beef meat	20	20	100
Chicken meat	31	26	83.9
Fish	20	16	80.0
Pork meat	24	23	95.8
Overall	95	85	89.5

3.2.2. Contamination Frequencies of Extended Spectrum β-lactamase *E. coli (ESBL_Ec)*

A total of 40 samples (42.1%) were contaminated by Extended Spectrum β-lactamase *E. coli* (Table 3) out of which beef meat and fish were the most contaminated samples with a frequency of 45. 0% (9/20) respectively, followed by chicken meat 41.9 (13/31). Pork meat was the least contaminated sample with a frequency of 37.5% (9/24). Khi-2 test results revealed that there was no significant difference of Extended Spectrum β-lactamase *E. coli* contamination among the different samples (khi-2 of Pearson: 0.38; p: 0.94 > 0.05). The distribution of in food samples is presented in Table 3.

Table 3. Contamination frequencies of Extended Spectrum β-lactamase *E. coli* (ESBL_Ec) contamination in collected samples

Sample type	Number of samples tested	Number of positive samples	Frequencies (%)
Beef meat	20	9	45. 0
Chicken meat	31	13	41. 9
Fish	20	9	45. 0
Pork meat	24	9	37. 5
Overall	95	40	42. 1

3.3. Antimicrobial Susceptibility Testing of *E. coli* Isolates

3.3.1. Phenotypic Resistance Pattern of β_glucuronidase Positive *Escherichia coli* Isolates

Beta_glucuronidase positive *Escherichia coli* isolates showed extremely high resistance rate for Amoxicillin, Amoxicillin/Clavulanic Acid and Ofloxacin (90. 9 % respectively) and Ticarcillin (81.8%), very high resistant rate was observed with chloramphenicol (63. 6%). Moderate resistance rate was observed with Imipenem, Amikacin and Tobramycin (10.2%, 18.2% and 27.3% respectively).

3.3.2. Phenotypic resistance pattern of extended spectrum β_lactamase *Escherichia coli* isolates

Extended spectrum β_lactamase *Escherichia coli* isolates expressed extremely high resistance rate to Amoxicillin, Amoxicillin/Clavulanic Acid, Cefotaxim, Ceftriaxone and Aztreonam (100% respectively). Extremely resistance rate was also observed with Ofloxacin (94. 7%) and Ticarcillin (81.8%). Very high resistance rate was observed with Nalidixic Acid and Ciprofloxacin and Tobramycin (45.5%, 63. 2% and 57.9% respectively). Moderate resistance rate was observed with Imipenem and Amikacin (18.2% and 26.3% respectively) (Table 4).

Table 4. Phenotypic resistance pattern of *E. coli* strains isolated from meat and fish products.

Antimicrobial	β-glucuronidase positive *Escherichia coli* (n= 60)			Extended spectrum β-lactamase *Escherichia coli* (n=19)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amoxicillin 20 µg	90.9	0.0	9.1	100.0	0.0	0.0
Amoxicillin/Clavulanic Acid 20/10 µg	90.9	0.0	9.1	100.0	0.0	0.0
Ticarcillin 75 µg	81.8	9.1	9.1	89.4	5.3	5.3
Cefotaxim 5 µg	100.0	0.0	0.0	100.0	0.0	0.0
Ceftriaxone 30 µg	100.0	0.0	0.0	100.0	0.0	0.0
Aztreonam 30 µg	100.0	0.0	0.0	89.5	0.0	10,5
Azithromycin 15 µg	27.3	0.0	72.7	36.8	0.0	63.2
Imipenem 10 µg	10.5	0.0	89.5	18.2	9.1	72.7
Chloramphenicol 30 µg	47.4	0.0	52.6	63.6	0.0	36.4
Amikacin 30 µg	18.2	0.0	81.8	26.3	0.0	73.7
Ofloxacin 5 µg	90.9	9.1	0.0	94.7	0.0	5.3
Tobramycin 10 µg	27.3	0.0	72.7	57.9	0.0	42.1
Nalidixic Acid 30 µg	45.5	0.0	54.5	63.2	0.0	36.8
Ciprofloxacin 5 µg	63.6	18.2	18.2	63.2	10.5	26.3

R: Resistant, I: Intermediate, S: Suceptible

3.4. Dissemination of Antimicrobial Resistance *E. coli* in Foods Samples

The dissemination of antimicrobial resistant *E. coli* in food samples was assessed using Antimicrobial resistance ratio (ARR). The results showed that chicken meat was the most disseminating product (ARR 63. 8%) followed by Fish (ARR: 59. 9%) and beef meat (ARR: 48. 1%). Pork meat was the least antimicrobial resistant disseminating product (ARR: 46. 0%). Khi-2 test results revealed that there was no significant difference among the ARR of the different strains of *E. coli* among the different samples ($p > 0.05$)

3.5. Multidrug Resistance (MDR) Patterns of *E. coli* Isolates

75 isolates (94. 9%) expressed resistance to at least three different classes of antimicrobial agents and were considered as multidrug resistant isolates. 34 isolates (43.0%) were resistant to 10 antibiotics classes with AMX+AMC+TIC+CTX+CRO+ATM+AZM+IMP+OFX +TOB as the main profile (19.0%), 18 isolates (22.8%) expressed resistance to 11 antibiotics classes with AMX+AMC+CTX+CRO+ATM+AZM+IMP+OFX+TOB +NAL+CIP as the main MDR profile (10.1%). Two isolates presented resistance to thirteen different classes of antimicrobial agents (Table 5).

Table 5. Frequencies of multi drug resistance *E. coli* isolates from meat and fish products

Multidrug resistant *Escherichia coli* Profile	Number of antibiotics	Number of isolates	frequencies of MDR *E. coli* (%)
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+OFX+TOB+NAL+CHL+CIP	13	2	2.53
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+OFX+TOB+NAL+CIP	12	4	5.1
AMX+AMC+CTX+CRO+ATM+AZM+ IMP+OFX+TOB+NAL+CIP	11	8	10.1
AMX+AMC+TIC+CRO+ATM+AZM+IM P+OFX+CHL+NAL+CIP	11	6	7.60
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+OFX+TOB+NAL	11	4	5.10
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+NAL+OFX+TOB	10	15	19.0
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+CHL+TOB	10	10	12.7
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+NAL+TOB	10	9	11.4
AMX+AMC+CTX+CRO+ATM+AZM+ IMP+OFX+TOB	9	3	3.80
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+IMP+OFX	9	3	3.80
AMX+AMC+TIC+CTX+CRO+ATM+ AZM+OFX	8	4	5.10
AMX+AMC+TIC+CTX+CRO+ATM+ AZM	7	5	6.33
AMX+AMC+TIC+CTX+CRO+ATM	6	3	3.80
AMX+AMC+TIC+CTX+CRO	5	2	2.53
AMX+AMC+TIC+CTX	4	1	1,27

AMX: Amoxicillin, AMC: Amoxicillin/Clavulanic Acid, TIC: Ticarcillin, CRO: Ceftriaxon, ATM: Aztreonam, AZM: Azithromycin, IMP: Imipenem, CHL: Chloramphenicol, AMK: Amikacin, OFX: Ofloxacin, TOB: Tobramycin, NAL: Nalidixic Acid, CIP: Ciprofloxacin

3.6. Distribution of Multidrug Resistance *E. coli* in Meat and Fish Products

27/28 isolates (96. 4%) from Chicken meat were MDR strains while 17/18 (94. 4%) isolates from fish, 17/18 (94. 9%) from pork meat and 14/15 (93. 3%) from Beef meat where consider as MDR. Khi-2 test revealed that there was not a significant difference on MDR *E. coli* distribution among the different samples.

Table 6. Frequencies of MDR *E. coli* from meat and fish products

Sample type	Number of isolates tested	Number of MDR isolates	Frequencies of MDR isolates (%)
Beef meat	15	14	93.3
Chicken meat	28	27	96.4
Fish	18	17	94.4
Pork meat	18	17	94.4
Total	79	75	94.9

4. Discussion

Monitoring the pathogenic *E. coli*, its antibiotic susceptibility profiles as well as its acquisition of virulence or resistance genes has become an important approach to understand the dynamism of antimicrobial resistance around the word, and particularly in developing countries like Cameroon, where there is a lack of data concerning drug resistant *E. coli* in circulation. Our study identified very high contamination rates (80% to 100%) of

β-glucuronidase positive *E. coli* from meat and fish products but not significantly different. This non-significant difference contrast with previous reports where significant frequencies of 33% and 55% were recorded on beef and chicken meats, respectively sampled in restaurants and sales points in Yaoundé [32,33]. The differences observed could either be due to the sampling method or to the experimental approach used. In our study we used a detection method with enrichment phase meanwhile in these studies, enumeration methods were used. According to Anderson et *al.* [34], enrichment culture is the technique that is used to enhance the population density of a particular group of microorganisms within the total microbial population of a sample. The high frequency of *E. coli* in meats and fish products may be the result of poor hygiene process during slaughtering. This result indicates an insufficient state of the sanitation process [4]. According to Uzeh Ekiomado et *al.* [35], initial exposure of meat to intestinal contents of food-processing animals during slaughtering leads to contamination of meat with *E. coli* which is currently the most widely used indicator of faecal contamination. Its presence indicates possible contamination with enteric pathogens and is often associated with poor sanitation or cross-contamination in food handling processes [36].

Studying pathogenic and antibiotic-resistant *E. coli* strains represents the best way to combat the spread of antimicrobial resistance genes that can be transferred to pathogenic or opportunistic bacteria [37]. *Escherichia coli* has been listed as a leading pathogen by the World Health Organization because of its widespread resistance to antibiotics [38]. Our study showed an average contamination rate of 42. 1% of meat and fish samples by Extended spectrum β-lactamase *E. coli* with beef meat as the most common source of contamination, followed by chicken meat and fish, with no significant differences between samples. These results are similar to those of Badr et *al.* [39] who found a prevalence of 46.7% on chicken meat in Egypt. However, our results differ from those of Djuikoue et *al.* [40] who observed a prevalence of 26.32% on broiler chickens in Yaounde. The observed differences may be due to differences in ESBL detection methods. In fact, in our study, ESBL *E. coli* were obtained in two steps, the first step involves screening for potential ESBL colonies on supplemented selective chromogenic media, followed by confirmation by double disk synergy assay, whereas in Djuikoue et *al.* [40] study, ESBL *E. coli* isolates were selected on methylene blue eosin agar and then confirmed by double disc synergy test. Previous studies have shown that chromogenic media are more sensitive and specific than ordinary media in detecting pathogens. *Escherichia coli* listed as a priority research pathogen by the World Health Organization is the most important gene pool encoding extended-spectrum β-lactamases [41]. The presence of extended spectrum β-lactamases *E. coli* in food samples intended for human consumption may indicate their continued widespread distribution from farm to fork continuum and poses a serious public health threat to human populations [42].

The presence of extended-spectrum β-lactamase *E. coli* strains in beef, pork, chicken meat, and fish samples could be explained by the uncontrolled use of antibiotics, especially third-generation cephalosporins, as growth promoters during breeding [43,44], lack of training programs for breeders on rational use of antibiotics and good breeding practices, and lack of national AMR guidelines and regulations. Furthermore, the high prevalence of ESBL *E. coli* in meat and fish products may be due to the lack of compliance with the international food safety standard ISO 22000:2018, lack of Hazard Analysis for Critical Control Points (HACCP) Compliance for abattoirs. Founou et *al.* [42] in previous studies in Cameroon abattoirs have pointed poor quality and lack of basic sanitation infrastructure as sources of ESBL *E. coli* contamination in our market.

Beta-glucuronidase positive and extended spectrum β-lactamase *E. coli* showed high multidrug resistance rate to tested antibiotics, although some of them were susceptible (Table 4). The resistance rate of ESBL *E. coli* to amoxicillin, amoxicillin + clavulanic acid, cefotaxime and ceftriaxone was 100%. The present results are in phase with the work of Ahmadi et *al.* [45] who reported that *E. coli* isolates from meat and meat products were resistant to amoxicillin, tetracycline, and other antibiotics. These results are also consistent with Messele et *al.* [46] who found similar antimicrobial resistance rate in *E. coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. Furthermore, Kapena et *al.* [47] also reported higher resistance rates in *E. coli* isolated from raw retail table eggs in Luzaka Zambia. The high resistance rates of our isolates to tetracyclines, aminoglycosides and penicillins could be explained by the fact that they are cheaper and widely used in breeding [48]. Numerous studies have shown high prevalence of multidrug-resistant *E. coli* in meat samples in Africa [49, 50]. The variability of resistance rate phenotypes in this study could be explained by the acquisition of resistance to several classes of antibiotics (co-resistance), as exchanged plasmids often have multiple resistance genes, such as those resistant to cephalosporins, penicillin, chloramphenicol, tetracycline, and fluoroquinolones [51]. These results could be explained as result of farmers' misuse of antimicrobials as growth promoters for prophylactic and post-prophylactic treatments. The abusive use of antibiotics as growth-promoting factors during farming and aquaculture causes the presence of residues in food of animal origin and the emergence of antimicrobial resistance [25,52].

In addition to the presence of antimicrobial resistant bacteria in the food chain, other consequences associated with the widespread use of antibiotics in aquaculture, livestock and crop production [53] are the resistance genes in stages [25,54] and human diseases [55]. Previous studies have reported high rates of drug residues in poultry meat in Cameroon and multidrug-resistant *E. coli* (including resistance to fluoroquinolones, carbapenems, and third-generation cephalosporins) in humans and animals [56,49,23]. There was no significant difference of antimicrobial resistance rate of our isolates toward Ticarcillin, Azithromycin, Imipenem, Chloramphenicol, Amikacin, Ofloxacin, Nalidixic Acid and Ciprofloxacin. Conversely a significant difference was observed with antimicrobial resistance patterns of our isolates toward Amoxicillin, Amoxicillin/clavulanic-acid, Cefotaxim, Ceftriaxon, Aztreonam and Tobramycin ($P<0,05$). Our study found that chicken meat was the leading source of

antimicrobial resistance dissemination through the food chain. These results could be explained by the high rate of use of antibiotics in chicken farming. The current results are consistent with the OIE's annual report on the use of antimicrobials in livestock. In Africa, cattle and poultry account for approximately half of antimicrobial consumption in animal production [57,58,59]. Resistance has been reported in a variety of MDR bacteria including WHO priority pathogens such as *E. coli* mainly in cattle and poultry [57,58,59]. As many African countries, Cameroon currently does not have antimicrobial use (AMU) and antimicrobial resistance (AMR) surveillance programs that can regulate antimicrobial use in farming and agriculture. Despite the above observations, the current study has some limitations, mainly due to the limited sample size of meat and fish products and the confirmation method of resistant *E. coli* only based on phenotypic and biochemical assays.

5. Conclusions

We conducted a cross-sectional study aimed at isolating and characterizing *E. coli* from fish and meat products collected from some retail markets in Douala, Cameroon. The results indicated that fish and meat products intended for human consumption were more likely to be contaminated with multidrug-resistant *E. coli* with higher occurrences. Antimicrobial susceptibility tests results revealed that various isolates were highly resistant to many antimicrobial classes. These indicate that chicken meat was the leading source of antimicrobial resistance dissemination through the food chain. These results are alarming and call for cautious use of antimicrobials in cattle and poultry farming. Therefore, it is necessary to implement hygiene guidelines in our markets and slaughterhouses and adhere to the HACCP system of biosecurity measures to create a better picture of the situation.

Authors Contributions

This manuscrit was written through contributions of all authors. All authors gave approval to the final version of the manuscrit.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This study does not involve human participants and thence does not require any ethical consideration.

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