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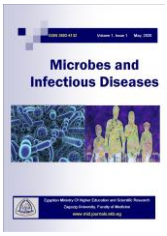

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Original article

Molecular characterization and multidrug resistance profile of extended spectrum beta lactamase producing *Escherichia coli* (ESBL) in poultry from Gusau metropolis, Nigeria

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ABSTRACT

Background: *Escherichia coli* causes life threatening disease in humans through ingestion of contaminated animal products such as milk and meat. Infections are widely distributed among poultry of all ages and categories and they are primarily related to poor hygienic conditions. **Methods**: A cross-sectional study was conducted to determine the presence of extended spectrum beta lactamase-producing *Escherichia coli* (ESBL) in chicken by conventional bacterial isolation, molecular characterization and drug sensitivity testing. Multi-drug resistant ESBL producing *Escherichia coli* (E. coli) were isolated from poultry (broilers, layers and chicken) from farms and live bird market in Gusau Metropolis, Nigeria using conventional methods of isolation, molecular characterization and antimicrobial susceptibility testing. **Results:** The overall prevalence of *E. coli* based on phenotypic identification was 22.1 % (64/289), and genotypic identification (PCR) was 12.1% (35/289). The overall prevalence of ESBL was 20% (6/30) while the prevalence of ESBL based on the chicken type was 37.5% (3/8), 18.7% (3/16) and 0% (0/6) for broilers, layers and local chickens respectively. Antibiotic susceptibility test revealed 88.5% resistance to amoxicillin-clavulanate and imipenem and 80% to cefuroxime, 62.8% erythromycin, 54.2% cefexime, 51.4% cefotaxime and azithromycin. However, 31.4% were susceptible to levofloxacin, 40% to ofloxacin, 45.7% to ceftriaxone, 45.7% to ciprofloxacin and 45.7% to gentamicin. **Conclusions:** the study revealed the prevalence of ESBLs *E. coli* 20% (6/30) and (*blaCTX-M*) gene in chicken in Gusau Metropolis and antibiotic resistance awareness is hereby recommended to be carried out by relevant agency.

Introduction

E. coli are gram negative bacteria that commonly inhabit the lower intestine of warm-blooded animals [1]. They cause serious illness in humans when ingested via contaminated animal products [2]. *E. coli* infections are common among poultry of all types and ages. Infections are primarily linked to poor hygiene [3]. Infection due to consumption of drug-resistant organisms in food animals like chicken has become a global threat to public health and require attention worldwide [4]. Genes associated with ESBL have been reported in Nigeria in different poultry species, especially chickens. The most common of the genes detected are *bla CTX-M* (Cefotaxime-Munich), *bla TEM* (Temoniera), and *bla SHV* (Sulphydryl Variable)

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[5]. A recent study by **Aworh et al.** reported another most common *bla-OXA* gene in Abuja, Nigeria.

Poultry production in Nigeria has been reported to accounts for 36.5 % of protein sources [6]. Diseases caused by antibiotic resistant ESBL *E. coli* can however hamper the poultry production and consequently lead to economic losses [7]. The contamination of chicken meat with ESBL *E. coli* harboring resistant genes at the abattoir and live-bird market during meat processing stages (slaughter, dressing, and evisceration) is a risk factor that could result in the spread of resistant ESBLs *E. coli* genes in humans [8]. Although poultry farming is getting more ground and the population of poultry farmers is rising in Gusau Metropolis, very little is known about the prevailing pathogens in poultry and their antimicrobial resistance potential in Zamfara State. It has been reported by World Bank that antimicrobial resistance will drastically impacts livestock production of low and middle income countries [9]. Developing sustainable guidelines for rational use of antimicrobials requires survey of the multidrug resistant organisms in food animals [10]. It has been reported that by 2050 up to 11% loss due to antimicrobial resistance may be incurred in livestock production [11]. This will result in a reduced income generation to farmers and an exacerbated poor economic situation [11]. More so, molecular techniques have proven to be more sensitive and reliable in disease diagnosis. Despite the high-performance characteristics of the method and the need to accurately diagnose diseases in animals, there is a dearth of information on the application of the technique in disease surveillance and diagnosis in the study area. Thus, there is a need to employ the molecular method of identifying ESBL *E. coli* and detect antibiotic resistance genes in the isolates from the study area. This study therefore aimed to investigate the presence of extended-spectrum-beta-lactamase-producing (ESBL) *Escherichia coli* in chickens within the Gusau metropolis, Zamfara State.

Material and methods

Study design, location and sample population

A cross-sectional study approach was carried out and samples were drawn between November 2020 to May 2021. Poultry farms and live bird markets were identified and convenient sampling was employed in selecting the sample sites while chickens were selected using simple random sampling from poultry houses. The targeted groups were broilers, layers, and local chickens. 55 sampling units were visited 22 broiler farms with a cumulative population of 12,000 were visited, 28 layer farms with a cumulative population of 218,720 were visited and five live bird markets in the metropolis with a population of 200 local chickens were visited.

Gusau is the capital of Zamfara State and the only Local Government Area in Gusau metropolis, It is located between latitude 12.1702 °N and longitude 6.6641°E and it occupies an area of 3,364km^2 (Allied Surveyors Topographic sheet, 1990). Based on the results of the 2006 National Population Census, Gusau Local Government Area has a population of about 682, 700 people [12]. About 82% of the population in the State is engaged in agriculture (livestock farming) on farms and some of the families do poultry production at home [13].

Sample collection

Cloacal swabs samples were collected using swab sticks from 40 broilers, 219 layers, and 30 local chickens during the study period. A disproportionate stratified random sampling was used in allocating the number of samples to the different chicken types (Layers, Broilers, and Local chickens) based on our study design in the different farms and live-bird markets in Gusau Metropolis. The samples were labeled accordingly, placed in buffered peptone water and carried in a cooler with an ice pack to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto and processed for the presence of *E. coli*.

Bacterial culture

Culture and isolation were carried out on nutrient broth and MacConkey agar (Oxoid, United Kingdom) by streak plate method. MacConkey agar was prepared according to the manufacturers' instructions. Lactose fermenters on MacConkey agar were streaked on Eosin methylene blue (EMB) agar media (Oxoid, UK). Identified *E. coli* isolates were kept on a nutrient agar slant (Oxoid, UK) at room temperature until use. Biochemical tests were conducted to phenotypically confirm the isolates as *E. coli*. All the media were prepared according to the manufacturer's instructions (Oxoid, UK). Gas production, hydrogen sulfide production and carbohydrates fermentation (glucose, lactose, Sucrose) were studied from pure colonies on MacConkey as described by [14, 15].

DNA extraction (Boiling method)

The DNA extraction was performed by boiling method as described previously by **Porteous et al** [16]. The slanted isolates were sub-cultured using nutrient agar at 37°C for 24 hours. A loopful of the *Escherichia coli* colonies was suspended in 100µl of nuclease-free water in a sterile microfuge tube and then mixed by vortexing. The tubes were properly sealed and centrifuged at 14,000 rpm for 3 min at room temperature. The supernatant was discarded while the cell sediments harvested were re-suspended in 100µl of sterile normal saline and mixed by vortexing for 15 seconds. The tubes were heated in a water bath at 90°C for 15 minutes and rapidly cooled by placing the tubes on ice for 10 minutes. Centrifugation was carried out at 14000 rpm for 1 min and the supernatant (DNA template) was transferred into a sterile microfuge tube and stored at -10°C until use.

Polymerase chain reaction (PCR) protocol for *E. coli* 16s gene

Polymerase chain reaction (PCR) was performed to amplify the ESBL 16S RNA gene [17]. A 25µl PCR reaction volume was prepared to contain 12.5µl of a master mix (Biolabs), 1µl of 16s forward primer (IDT®), 1µl of 16s reverse primer (IDT®), 1µl of nuclease energy-free water (Biolabs), 1.5µl coral load (Biolabs) and 8µl of DNA template.

The PCR amplification comprises an initial denaturation step at 94℃ for 3 minutes and 40 cycles of denaturation at 94℃ for 30 seconds, annealing at 54℃ for 30 seconds and extension at 72℃for 1 minute, then final extension at 72℃ for 10 minutes.

PCR amplification of *bla-CTX-M, bla-SHV,* and *bla-TEM* genes

Multiplex polymerase chain reaction (PCR) was performed to amplify the *bla-CTX-M, bla-SHV* and *bla-TEM* genes. The PCR was performed in a 25µl reaction volume consisting of 12.5µl of a master mix (Biolabs), 0.5µl of *SHV* gene forward primer (IDT®), 0.5µl of *SHV* gene reverse primer (IDT®), 0.5µl of *CTX* gene forward primer (IDT®), 0.5µl of *CTX* gene reverse primer (IDT®), 0.5µl of *TEM* gene forward primer (IDT®), 0.5µl of *TEM* gene reverse primer (IDT®), 0.5µl of nuclease energy-free water (Biolabs), 1µl coral load (Biolabs), and 8µl of DNA template. The PCR was also performed using the DNA amplification method comprising an initial denaturation step at 94℃ for 5 minutes, 40 cycles of denaturation at 94℃ for 30 seconds, annealing at 56℃ for 40 seconds, and extension 72℃ for 50 seconds and final extension at 72℃ for 10 minutes. The PCR products were loaded in 1% agarose gel (Vivantis Incorp, USA), 1x TBE (Vivantis Incorp, USA) buffer (Tris 0.09M- borate 0.09M-EDTA 0.02M) pre-stained with ethidium bromide (Biotium, Hayward, USA) [18].

Electrophoresis was carried out at 70 volts for 70 minutes (Thermo Owl Separation Systems, USA). Molecular markers of 100bp (GeneDirex, Taiwan) were run in parallel well with the DNA samples as an indicator of the size of PCR amplicons. Gel was visualized under a UV light trans-illuminator (Major Science, USA), and the images were taken.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done using the Kirby-Bauer disc diffusion method. Twelve (12) antibiotics comprising cefuroxime 30µg, cefixime 5µg, cefotaxime 25µg, ceftriaxone sulbactam 45µg, levofloxacin 5µg, ciprofloxacin 5µg, amoxicillin- clavulanate 30µg, ofloxacin 5µg, erythromycin 15 µg gentamicin 10µg, azithromycin 15 µg and imipenem 10µg (Celtech Diagnostic BDR003) were used based on Clinical Laboratory Standard Institute guidelines [19]. Colonies of *Escherichia coli* isolates were inoculated into 5 ml of Mueller-Hinton broth (MHB) and incubated at 37ºC for 24 hours. After overnight incubation at 37°C, pure colonies were suspended in 400µl of sterilized distilled water, and turbidity was adjusted to 0.5 McFarland at 600 nm wavelength using a spectrophotometer. A spread plate inoculation technique was used; a lawn of bacteria was streaked on inoculated Mueller-Hinton agar plates using a sterile swab stick. Antibiotic discs were placed gently on the Mueller Hinton agar plates using a pair of sterile forceps and the plates were incubated for 24 hours at 37ºC. After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler, and the result of the susceptibility test was interpreted using susceptibility breakpoint as described by [19].

Statistical analysis

The data obtained from the study were summarized and presented in tables and charts. The chi-Square test was used to determine the association between some variables and the presence of ESBL. A 95% confidence interval and

5% level of significance were used. The analysis was done using SPSS version 23.0.

Results

From the study, the overall prevalence of ESBL was 20% (6/30) **(Table 1)**. The prevalence of ESBL *E. coli* based on the chicken type was 37.5% (3/8), 18.7% (3/16) (5 samples deteriorated), and 0% (0/6) for broilers, layers, and local chickens respectively. There was no statistically significant association between the presence of the gene (*bla CTX-M*) and the type of chicken (***p*** = 0.2838; DF = 2) **(Table 2)**. Antibiotic susceptibility test using 12 antibiotics from four different classes of antimicrobials drugs revealed 88.5% resistance to amoxicillin-clavulanate and imipenem, 80% to cefuroxime, 62.8% to erythromycin, 54.2% to cefexime, 51.4% to cefotaxime and azithromycin. However, 31.4% of the isolates were susceptible to levofloxacin, 40% to ofloxacin, 45.7% to ceftriaxone, 45.7% to ciprofloxacin and 45.7% to gentamicin **(Table 3)**. The antimicrobial resistance profile of the *E. coli* isolated from layers revealed high resistance in 54.2% of the isolates to amoxicillin-clavulanate, 51.4% to imipenem, 42.8% to cefuroxime, 40% to erythromycin, 34.2% to cefotaxime. The antimicrobial resistance profile of *E. coli* isolated from broilers revealed high resistance in 22.8% of the isolates to cefuroxime and imipenem respectively, and 20% to erythromycin and amoxicillin-clavulanate respectively. The antimicrobial resistance profile of *E. coli* isolated from local chickens revealed high resistance in 17.1% of the isolates to amoxicillin-clavulanate, cefuroxime, and imipenem respectively **(Table 4)**.

Table 1. Prevalence *E. coli,* ESBL and Presence of *bla-CTX-M* gene in ESBL producing *E. coli* isolated from different chicken types

Type of chicken	Isolates (No.) *E. coli*	ESBL *Bla CTX-M* gene
Broilers	8 20% (8/40)	37.5% (3/8)
Layers	16 9.58% (21/219)	18.7% (3/16)
Local chickens	6 20% (6/30)	0% (0/6)
Total	30	20% (6/30)
Overall prevalence	22.1% (64/289)	12.1% (35/289)

Key: CTX-M: Cefotaxime- Munich, *Bla*: Beta- lactamase

Table 2. Univariable analysis for the presence of *bla-CTX-M* gene in ESBL *E. coli* isolated from different chicken types

Chicken types	Negative	Positive	Total	***p***- value	DF*
Broilers	5	3	8		
Layers	13	3	16		
Local chickens	6	0	6		
Total	24	6	30	0.2838	2

Key: DF: Degree of freedom

Table 3. Total antimicrobial resistance profile of *Escherichia coli* isolated from broilers, layers and local chickens

Antibiotics	Layers n=21	Broilers n=8	Local chickens=6	Total n=35	% Resistance
Cefuroxime	L15	B8	LC5	28	80.0
Cefexime	L10	B6	LC3	19	54.2
Cefotaxime	L11	B6	LC1	18	51.4
Ceftriaxone	L8	B6	LC2	16	45.7
Levofloxacin	L6	B4	LC1	11	31.4
Ciprofloxacin	L9	B6	LC1	16	45.7
Amoxicillin clavulanate	L18	B7	LC6	31	88.5
Ofloxacin	L7	B5	LC2	14	40.0
Erythromycin	L12	B7	LC3	22	62.8
Gentamycin	L8	B6	LC2	16	45.7
Azithromycin	L9	B6	LC3	18	51.4
Imipenem	L18	B8	LC5	29	88.5

Table 4. Antimicrobial resistance profile of *Escherichia coli* isolated from layers, broilers and local chickens (n = 35)

Antibiotics	Break point (mm)	Resistant (%), No. Layers	Resistant (%), No. Broilers	Resistant (%), No. Local chickens
Cefuroxime	≥ 23	L15(42.8)	B8(22.8)	LC6(17.1)
Cefexime	≥19	L10(28.5)	B6(17.1)	LC3(8.57)
Cefotaxime	≥23	L12(34.2)	B6(17.1)	LC1(2.85)
Ceftriaxone	≥21	L9(25.7)	B6(17.1)	LC2(5.71)
Levofloxacin	≥17	L6(17.1)	B4(11.4)	LC1(2.85)
Ciprofloxacin	≥21	L9(25.7)	B6(17.1)	LC1(2.85)
Amoxicillin clavulanate	≥18	L19(54.2)	B7(20.0)	LC6(17.1)
Ofloxacin	≥16	L7(20.0)	B5(14.2)	LC2(5.71)
Erythromycin	≥23	L14(40.0)	B7(20.0)	LC3(8.57)
Gentamycin	≥15	L8(25.8)	B6(17.1)	LC2(5.71)
Azithromycin	≥18	L9(25.7)	B6(17.1)	LC3(8.57)
Imipenem	≥16	L18(51.4)	B8(22.8)	LC6(17.1)

Table 5. Multiple antibiotic resistance (MAR) Index of *E. coli* isolated from layer, broiler and local chickens in Gusau, Zamfara State

No. of antibiotics isolate is resistant to (a_1)	No. of antibiotics isolate is resistant to (a_2)	No. of antibiotics isolate is resistant to (a_3)	No. of antibiotics tested (b)	MAR index (a_1/b)	MAR index (a_2/b)	MAR index (a_3/b)
Layers	Broilers	Local chickens		Layers	Broilers	Local chickens
L8	B9	LC8	12	L0.6	B0.7	LC0.6
L8	B12	LC7	12	L0.6	B1	LC0.5
L8	B12	LC6	12	L0.6	B1	LC0.5
L9	B11	LC3	12	L0.7	B0.9	LC0.2
L9	B7	LC9	12	L0.7	B0.5	LC0.7
L8	B4	LC4	12	L0.6	B0.3	LC0.3
L5	B8	_	12	L0.4	B0.6	_
L5	B12	_	12	L0.4	B1	_
L6	_	_	12	L0.5	_	_
L3	_	_	12	L0.2	_	_
L10	_	_	12	L0.8	_	_
L8	_	_	12	L0.6	_	_
L4	_	_	12	L0.3	_	_
L6	_	_	12	L0.5	_	_
L12	_	_	12	L1	_	_
L8	_	_	12	L0.6	_	_
L4	_	_	12	L0.3	_	_
L6	_	_	12	L0.5	_	_
L3	_	_	12	L0.2	_	_
L3	_	_	12	L0.2	_	_
L3	_	_	12	L0.2	_	_

Table 6. Pattern of Multi-Drug Resistance of ESBL-producing *E. coli* isolated from different chickens types

No. Agents Resisted	No. (%) isolates (n=35)	Multidrug resistant pattern
Broilers		
B2	2(5.71)	CXM, ERY
B2	2(5.71)	CXM, IMP
B3	3(8.57)	IMP, AUG, ERY
B3	3(8.57)	CXM, ERY, IPM
B4	4(11.4)	CXM, AUG, IMP, ERY
Layers		
L2	1(2.85)	CXM, ERY
L2	1(2.85)	CXM, IMP
L3	2(5.71)	IMP, AUG, ERY
L3	2(5.71)	CXM, ERY, IPM
L4	1(2.85)	CXM, AUG, IMP, ERY
Local chickens		
LC2	2(5.71)	CXM, ERY
LC2	2(5.71)	CXM, IMP
LC3	3(8.57)	IMP, AUG, ERY
LC3	3(8.57)	CXM, ERY, IPM
LC4	4(11.4)	CXM, AUG, IMP, ERY

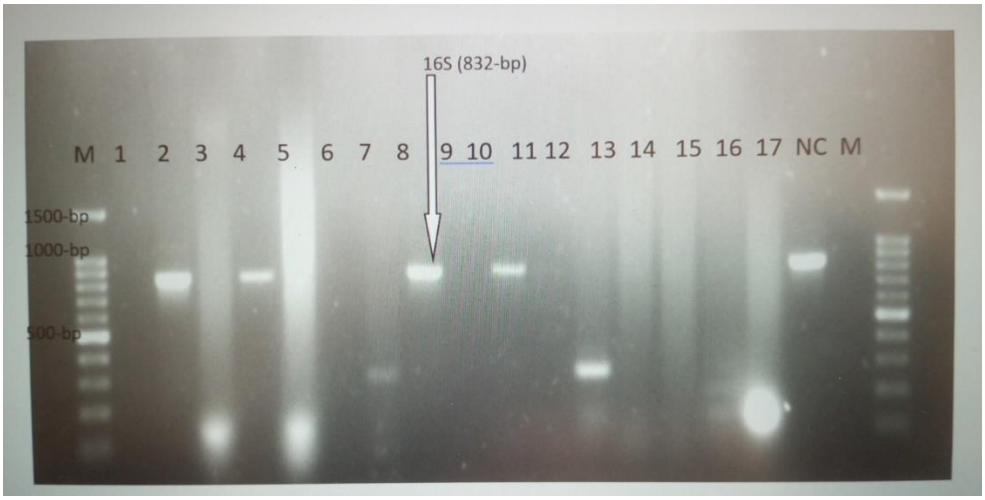
Key: CXM: Cefuroxime, ERY: Erythromycin, IMP: Imipenem, AUG: Amoxicillin-clavulanate

Figure 1. *E. coli* on Eosin methylene blue agar

Figure 2. Biochemical tests

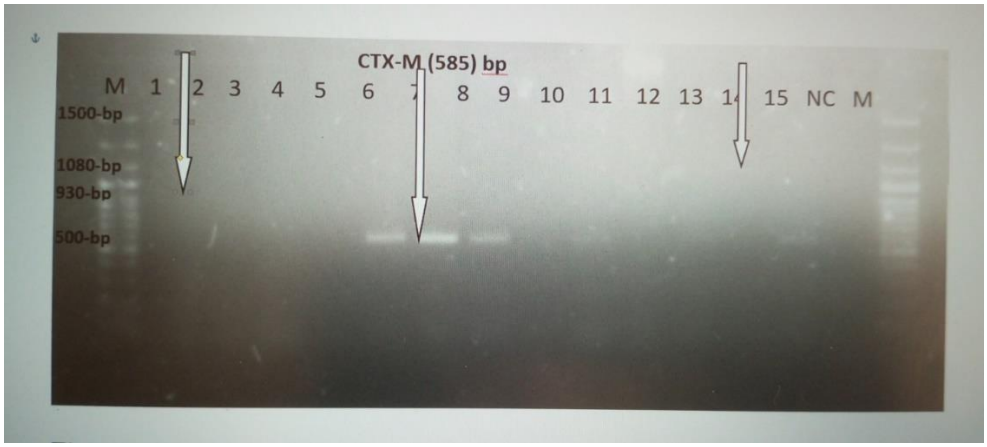
Figure 3. Sensitivity test

Figure 4. Agarose gel image showing bands of the amplified 832bp ESBL *E. coli* gene (16s) fragment



Key: M- Molecular ladder, well numbers: 2-17, positive ESBL *E. coli* gene (16s), NC-negative control

Figure 5. Agarose gel image showing bands of the amplified 585bp ESBL *E. coli CTX-M* gene fragments and none in the *SHV* 930bp and *TEM* 1080bp



Key: M- Molecular ladder, well numbers: 6-15 is the positive ESBL *E. coli* genes (*CTX-M*), NC-Negative control

Discussion

Food-producing animals including chicken are contaminated by bacteria and this usually spread through food chain. For this reason, chicken serve as an important reservoir for antimicrobial resistance genes of *E. coli* and this poses a significant risk of transmission to humans [20,21]. A survey on ESBL-producing *E. coli* in chicken has not been fully conducted in Zamfara State, Nigeria.

The overall prevalence of *E. coli* based on genotypic identification was 12.1% (35/289). The overall prevalence of ESBL was 20% (6/30). The prevalence of ESBL in this study is similar to that of **Chishimba et al** [22] who reported 20.1% prevalence among chickens in Zambia. The high prevalence observed may be due to the frequent serving of antimicrobials to chickens for treatment and prophylaxis [9]. The reason for the highest prevalence in broilers could be linked to high exposure to antibiotics for treatment and prophylaxis which might result in the selection of drug-resistant bacteria [23], while in local chickens it could be from the environment or cross-transmission from other species of animals in close contact as described [24]. In this study, the lowest prevalence of *E. coli* (9.58%) in layers was observed, which supports the report of **Mamza et al** [25] with a prevalence of (10.3%) in layers in Maiduguri. The reason for the lowest prevalence in both studies could probably be because of the good sanitary conditions of the environment where the two studies source the samples which in turn can be a risk of contamination with *E. coli* [26]. There was no statistically significant association between the detection of ESBL *E. coli* and chicken type ($\boldsymbol{p}$ = 0.0579; DF = 2). This means that all chicken types have an equal chance of acquiring the pathogen when exposed to it. This is further buttressed by the findings of [7].

All the ESBL-producing *E. coli* recovered were negative for *bla-TEM* and *bla-SHV* resistance genes but 6 (20%) were positive for the *bla-CTX-M* gene. The prevalence of ESBL based on the chicken type was 37.5% (3/8), 18.7% (3/16) (five samples from layers deteriorated because they were stored for a long time, thus sixteen were used for the PCR instead of twenty-one), 0% (0/6) for broilers, layers, and local chickens respectively. There was no statistically significant association between the occurrence of the *bla-CTX-M* gene in the ESBL-producing *E. coli* and chicken type ($\boldsymbol{p}$ = 0.2838). This is in line with the findings of **Murtaza et al** [27] where only *bla-CTX-M* was detected with a positive proportion rate of 10.7%. This could probably be because, since early 2000, the *CTX-M* group has taken over as the most common ESBL type in Enterobacteriaceae [28].

The multiple antibiotics resistance (MAR) indexes is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to [29]. A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used which has a formula; MAR=a/b where a= number of the antibiotic isolate is resistant to, and b= number of antibiotics tested. MAR index is an effective, valid, and cost-effective method that is used in the source tracking of antibiotic-resistant organisms [30]. Multiple drug resistance (MDR) or multidrug resistance is the antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial classes.

The multiple antibiotics resistance index (MAR) is the ratio between the number of antibiotic classes an isolate is resistant to and the total number of antibiotic classes the organism is exposed to [17]. The MAR index of 21 *E. coli* isolates from layers revealed 4 to have a MAR index of 0.2, while 17 isolates have values greater than 0.2. In broilers, all 8 isolates have a value greater than 0.2, while 5 isolates from local chickens have a value greater than 0.2. MAR indexing provides a useful tool for better risk assessment by identifying contamination from high-risk environments [31]. In this study, the MAR index of all the ESBL *E. coli* isolates is greater than or equal to 0.2 which differs from the study by **Tama et al** [17] where he obtained two (2.66%) isolates of less than 0.2 suggesting that most of the isolates originated from live bird markets and chicken farms where antibiotics were freely abused or misused in our study.

Multidrug resistance is defined as the resistance of an isolate to at least three antimicrobial agent from different antimicrobial classes [11]. Four different antimicrobial drugs were combined in the pattern; cefuroxime, erythromycin, imipenem, and amoxicillin-clavulanate. Another method that is often used by various researchers to characterize organisms as MDR is based on in vitro antimicrobial susceptibility test results when researchers tested resistance to multiple antimicrobial agents, classes, or subclasses of antimicrobial agents [32]. An overview of this variability of definitions is given in a report by **Sweeney et al** [33] which is used as a reference by some researchers. The MDR in this study is similar to that of **Eze et al** [34] who reported high resistance rates of the ESBL-positive *E. coli* isolates to tested antibiotics. In the production of chickens on farms, high concentrations of microorganisms in the air often occur in the environment in cages [18]. These microbes in such an environment can survive in the form of aerosols for a long time in the air and be transmitted with airflow [35]. Previous studies have shown the spread of ESBL-producing *E. coli* from the surrounding area [36]. The human population can be exposed to antimicrobial-resistant bacteria through encounter interactions with poultry sold in live bird markets, which are the source of the presence of MDR and ESBL bacteria [37].

Conclusion

The results from this study showed that ESBL *E. coli* were prevalent with an overall prevalence of 12.1% in chickens from poultry farms and live bird markets in Gusau metropolis, Zamfara State. There was no statistically significant association between the presence of ESBL-producing *E. coli* and chicken type. None of the ESBL-producing *E. coli* was seen to harbor *bla-SHV* and *bla-TEM* genes, while only (20%) 6 isolates out of 35 isolates had *bla-CTX-M* gene which can lead to risk of transfer of the genes to humans.

Recommendations

The *E. coli* isolates in our studies can be sequenced to fully understand the genetic variability between isolates. There should be public awareness and regulations by relevant agencies like the Veterinary Council of Nigeria (VCN) on how to appropriately give antibiotics and other drugs to chickens and humans to avoid antimicrobial resistance. Further studies should be carried out using other antibiotics to determine the MAR and MDR.

Conflict of interest

None

Funding

None

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