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Prevalence and Risk Factors of Extended-spectrum β-Lactamase-Producing *Escherichia coli* from Poultry Flocks in Benin City, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

People working in proximity to chickens farms or markets are at greater risk of developing antimicrobial resistance from Extended-Spectrum Beta-Lactamase-producing *Escherichia coli* (ESBL-EC) due to its zoonotic potential. The aim of this study was to determine the prevalence and identify the risk factors associated with ESBL producing *E. coli* from poultry farm environments across Benin City, Edo State. This Study was conducted at the University of Benin Teaching Hospital in Benin City, Nigeria. A total of 400 isolates of *Escherichia coli* was obtained from poultry birds' faeces. Identification of the isolates was done using Standard bacteriological techniques. Antimicrobial susceptibility test was performed using Kirby-Bauer diffusion method. ESBL production by isolates was detected by the method of Double Disc Synergy Test (DDST). The statistical tool used in this study was Chi-square test ($p \leq 0.05$ was set for significance). A prevalence rate of 21% ESBL production was detected in the400 isolates of *Escherichia coli*. The detection rate of ESBL producing *E. coli* was higher in Hen (28.1%) than in Cocks (17.6%), and comparably higher in poultry birds aged 13-17 weeks old (29%, $p = 0.00032$). No statistically significant differences were observed between housing system and frequency of antibiotic treatment. The ESBL-producers were most frequently detected in the frequency of the Antibiotic treatment (40%). ESBL-*E. coli* and non ESBL-*E. coli* producers were susceptible to Gentamicin and Augmentin. Moreover, ESBL- *E. coli* producers had a lower susceptibility profile compared to non ESBL- *E. coli* producers. Conclusively, the introduction of ESBL-producing *E. coli* from poultry farms to the environment may pose a potential health risk if these bacteria reach places where people may become exposed. The relatively high prevalence of ESBL-producing *E. coli* recorded in this study calls for routine detection, surveillance, and stricter regulations on antibiotic use among poultry producers in Nigeria.

Keywords: Antimicrobial resistance; extended-spectrum beta-lactamase; antibiotics; Escherichia coli.

1. INTRODUCTION

"The widespread use of antibiotics in food animal production has resulted in the emergence of antimicrobial-resistant bacteria that can be transmitted to humans not only via the food chain but also in the environment,e.g., in surfacewater and soil" (Kummereret al*.,* 2004). "A particular kind of antibiotics resistant that presently addresses a significant general wellbeing concern is the third-generation cephalosporin resistant incited by Extended spectrum Beta-lactamase (ESBL) production" (Canton et al*.,* 2008). "Antimicrobial resistance (AMR) in current times has been a serious issue and has gained global awareness resulting to the multi-drug (MDR) resistant organisms such as antimicrobial-resistant *Escherichia coli"* (Gbononet al., 2018). "A specific type of antibiotic resistance that currently represents a major public health concern is the third-generation cephalosporin resistance induced by Extended spectrum Beta-lactamase (ESBL) production" (Canton et al., 2008; Nakano et al*.,* 2023).

"Bacteria that bring about ESBL are resistant to not entirely all beta-lactam antibiotics, and usually to other classes of antibiotics as well, which results in challenges to treat infections, and additionally force the use of so-called last resort antibiotics, e.g., carbapenems, resulting in accelerated resistance to these types of antibiotics" (Canton et al., 2012). "Primarily, ESBL-production was mainly observed in hospitals infections caused by *Klebsiella*

pneumoniae, and mostly urinary tract infection caused by *Escherichia coli"* (Livermore et al., 2007).

In community patients and healthy individuals, a prevalence of ESBL-producing Entero-bacteriaceae of 5% - 10% has been described. Huijberset al., (2013) which in the study on community patients, species identified, were shown to be primarily *Escherichia coli* (Reulandet al., 2013). "The future threat of increased occurrence of untreatable infections requires mitigation of dissemination routes. Spread of ESBL-producing *Escherichia coli* in the community maybe facilitated by direct contact with human carriers, but alternatively, may also be livestock-related. ESBL-producing *Escherichia coli* were detected on 100% of Dutch broiler farms studied" (Dierikxet al., 2013). "The high prevalence of ESBL-producing *Escherichia coli* on Dutch retail chicken meat, and overlap between ESBL-genotypes from chicken meat and clinical *Escherichia coli* isolates, has led to the suggestion of chicken meat as a source of ESBL-producing *Escherichia coli*. Antibiotics resistant intestinal bacteria end up in the environment with animal and human feces. A major human contamination source is wastewater, either discharged onto surface water after treatment by wastewater treatment plants or discharged untreated through sewage overflows during heavy rainfall" (Dierikxet al., 2013). Examples of animal environmental contamination sources are animal manure used for field application and livestock farms(Blaaket al., 2014). "At livestock farms, bacteria may enter the natural environment (i.e. ambient air, soil, surface water) directly with droppings of pasture animals and free-range animals, or indirectly from barns, for instance through air and dust, with hands or feet of farm workers. Once in the environment, the bacteria may spread further away from farms with motile environmental compartments such as air and surface water, where people may get exposed to them, for instance through inhalation during recreation in down-stream located surface water, or when down-stream located water is used for irrigation of crops" (Blaaket al., 2014). "An additional route of dissemination of ESBL-producing *Escherichia coli* from farms may be with pest animals, e.g., flies, which have been recognized as transmitters of infectious diseases" (Greenberg et al., 1973). "Flies may move from farms where they were bred in, and have fed on, feces and carcasses to next feed on food meant for human consumption, ESBL-producing *Escherichia coli* in the poultry farm environment" (Nazniet al., 2005). Hence this study is to determine the extent of contamination of poultry farms with ESBL-producing *Escherichia coli* strains in Benin City, Nigeria.

2. MATERIALS AND METHODS

The cross-sectional study was carried out in the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin city, Nigeria. A total of 400 fecal samples were collected from both broilers and layers poultry birds in Benin City, Nigeria. The Medical Microbiology Laboratory at the University of Benin Teaching Hospital (UBTH), Benin City, received these samples for culturing and susceptibility testing. The isolates were identified using the standard microbiological technique described by Aflakianet al., (2022). including Colonial Morphology, wet preparation, Gram Stain, Indole Test, Simmons Citrate Test, Christensen's Urease Test, Methyl red, Voges-Proskauer test, and Motility Test. All isolates were kept at -70°C in trypticase soy broth with 15% (v/v).

The culture media used for culturing and identification include MacConkey agar, blood agar, and Muller Hilton Agar. The counting of viable colonies was done manually by examining the plates under Sui-Figure lightning. Antibiotic sensitivity testing was performed using the Kirby-Bauer disc diffusion technique as recommended by (CLSI 2020) for the following disks: Amoxicillin clavulanate (30ug), Cefotaxime (30ug), Ceftazidime (30ug), Septrin(30ug), Gentamicin (30ug), Pefloxacin (30ug), and Ofloxacin (30ug), and the presence of ESBL in all isolates was detected using the double disc synergy test, as described by Livermore and Brown (2001).

Socio demographic data accompanying the specimens, such as type of birds, age, gender, housing, if bird is on medication were obtained from the poultry farm workers. Cultured and identified colonies of Escherichia coli were used for this survey.

2.1 Statistical Analysis

The data obtained were analyzed with Chi square (x^2) using the statistical software INSTAT (Graph and software inc, LA Jolla, CA, USA). A p-value of less than 0.05 was considered significant.

3. RESULTS

Table 1 showed that Female which are the Hen had 36 positive ESBL producing *Escherichia coli* while Male which are the Cock had 48 positive ESBL producing *Escherichia coli*. ESBL production in relation to Gender of Poultry birds, and prevalence of ESBL production was not statistically significant (P = 0.125952).

In Table 1, the variable prevalence based on age showed that the Chickens aged between 13-17 weeks showing the highest prevalence of 29% while Chickens aged 6-9 weeks showed the least prevalence of 10.5%. The prevalence of ESBL production in relation to age was statistically significant (P = 0.00032).

Table 1 showed that the highest number of ESBL producing *Escherichia coli* was the Battery cage housing system. The prevalence of ESBL production in relation to Housing system was statistically not significant (P = 0.125952).

Table 1 showed the prevalence of ESBL producing *Escherichia coli* in relation to frequency of Antibiotics use and prevalence of ESBL production was statistically not significant (P = 0.085358).

Table 1 showed the prevalence of ESBL producing *Escherichia coli* in relation to Type of birds and prevalence of ESBL production was not statistically significant (P = 0.193587).

Fig.1 showed the general susceptibility profile of faecal*Escherichia coli. Escherichia coli* was sensitive to the respective antibiotics but its sensitivity was highest among Ceftazidime, Cefotaxime, and Gentamicin.

Fig.2 showed that the susceptibility profile of ESBL-producing *Escherichia coli*, in which ESBL-producing *Escherichia coli* showed low susceptibility to the respective antibiotics used except for Gentamicin which ESBL-producing *Escherichia coli* showed had sensitivity of about 21%

Fig.3 showed the susceptibility profile of non-ESBL producing *Escherichia coli*. All antibiotics were sensitive with Gentamicin showing the highest sensitivity of about of 56%.

Table 1. Distribution of ESBL enzymes in relation to Social – demographic factors of poultry birds

Category	Factor	No of *E.coli* tested	ESBL Positive (%)	p-value
Gender	Hen	128	36 (28.1)	0.125952
	Cock	272	48 (17.6)	
Age (weeks)	6-9	76	8 (10.5)	0.00032
	10-12	88	16 (18.2)	
	13-17	124	36 (29)	
	≥18	112	24 (21.4)	
Housing	Deep litter system	120	28 (23.3)	0.125952
	Battery cage system	280	56 (20)	
Frequency of Antibiotic Treatment	Weekly	120	48 (40)	0.085358
	Bi-weekly	60	20 (33.3)	
	Monthly	220	16 (7.27)	
Type of Birds	Broilers	340	72 (21.2)	0.193587
	Layers	60	12 (20)	

ESBL- Extended spectrum Beta-lactamase, *E. coli* - *Escherichia coli*:

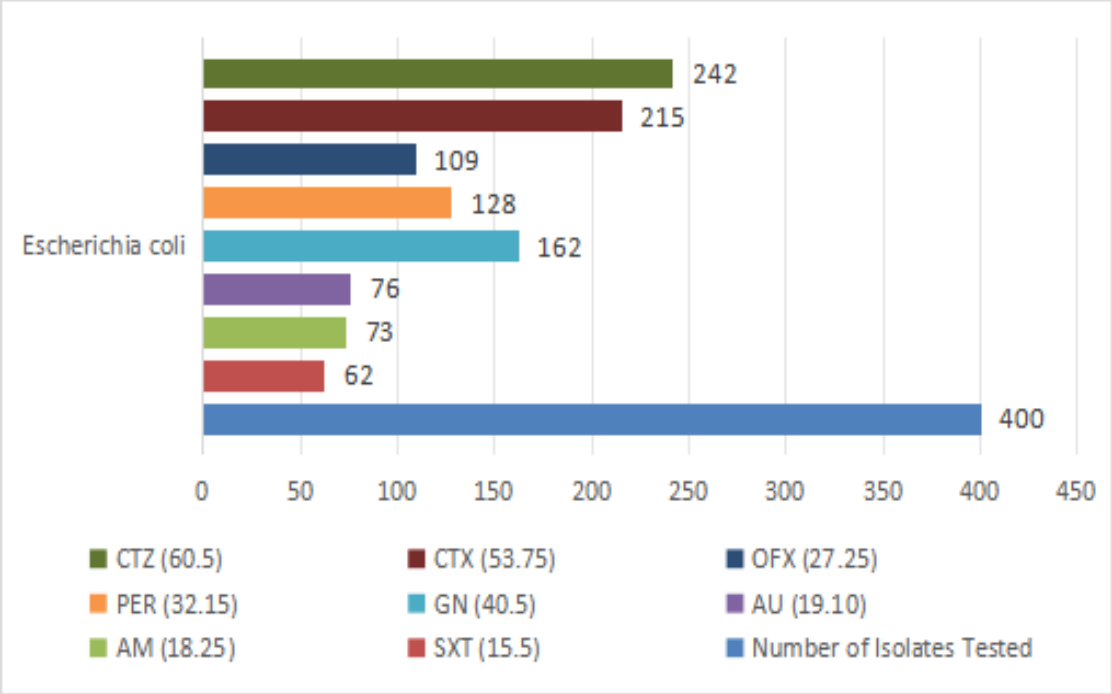

Fig. 1. Susceptibility profile of fecal *Escherichia coli*

SXT = Trimethoprim and sulfamethoxazole AU = Amoxicillin clavulanate, GN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin,. CTX= Cefotaxime. CTZ= Ceftazidime

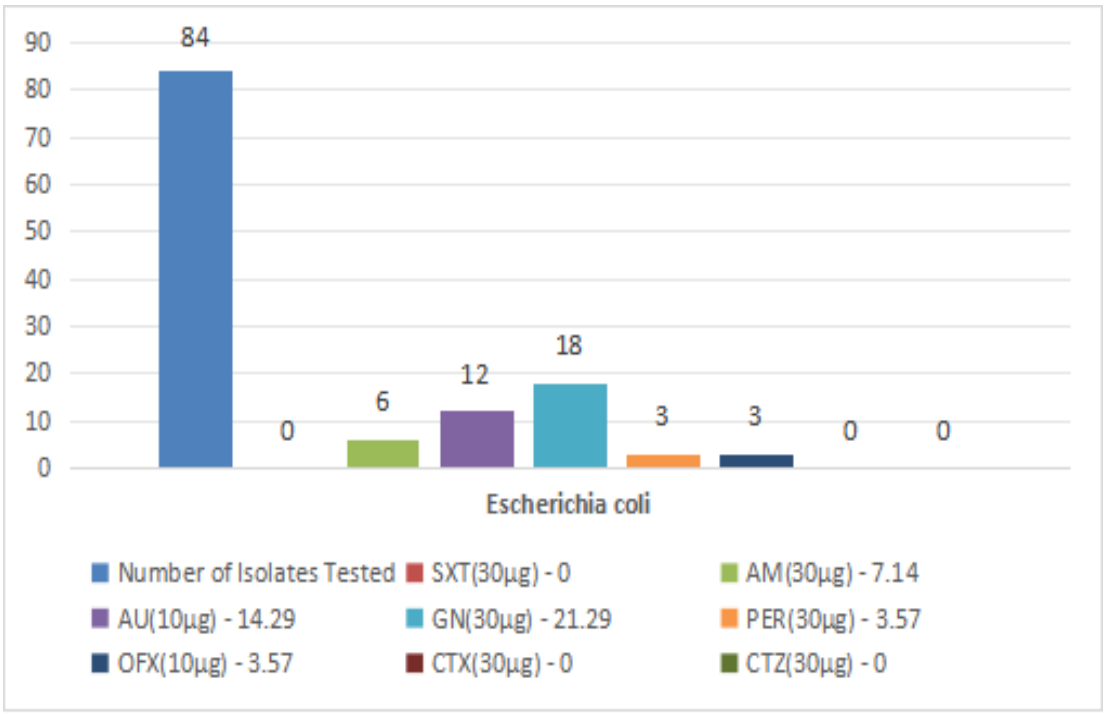

Fig. 2. Susceptibility profile of ESBL positive fecal *Escherichia coli*

SXT = Trimethoprim and sulfamethoxazole AU = Amoxicillin clavulanate, GN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin,. CTX= Cefotaxime. CTZ= Ceftazidime

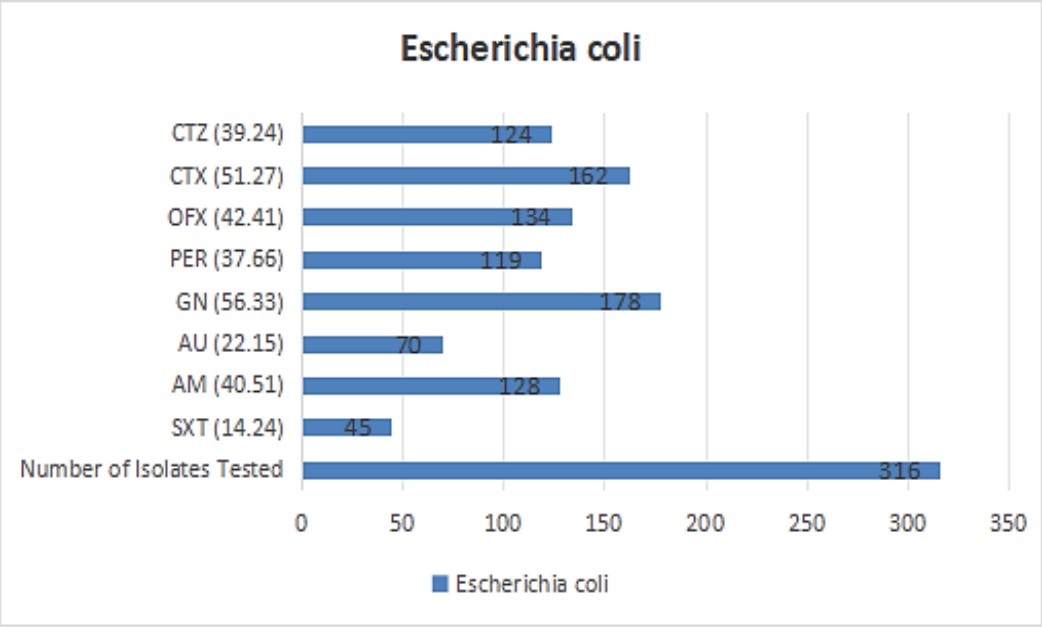

Fig. 3. Susceptibility profile of non-ESBL producing fecal *Escherichia coli*

SXT = Trimethoprim and sulfamethoxazole AU = Amoxicillin clavulanate, GN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin,. CTX= Cefotaxime. CTZ= Ceftazidime

4. DISCUSSION

A total of 400 fecal samples was collected from chickens in various poultry farms in Benin City and screened for the presence of ESBL-prosducing *Escherichia coli*. "The prevalence of ESBL-producing organisms has been increasing rapidly worldwide. This situation is alarming because ESBL producers have been reported to exhibit co-resistance to many other classes of antibiotics resulting in limited therapeutic options" (Nathisuwan et al., 2001; Liu et al. 2016 Mbiakopet al*.,* 2023) and increased morbidity and mortality (Husnaet al., 2023). In this study, the overall prevalence of ESBL producing isolates from 400 isolates of *Escherichia coli* was 21%. Higher prevalence rates of 29%, 32.2%, have been reported (Falgenhauer et al., 2019; Mabel et al., 2020). Other reports show that Pakistan (Riaz et al., 2012), Nigeria (Aworh et al., 2020), and India (Rao et al., 2014) and recorded 29.45%, 37.8%, and 57.5% respectively. "The variation in ESBLs prevalence rates reported between geographical areas, institutions, and countries may be attributed to the complex epidemiology of ESBLs, specific type of bacteria involved and methods used for ESBL detection among other factors" (Al Jasser, 2006; Kaur et al., 2013).

The highest occurrence of ESBL producing *E. coli* (40%) was observed in poultry birds given antibiotic treatment weekly. The findings of this study also showed that the occurrence of *E. coli i*n different age range of poultry birds, with the highest prevalence of ESBL producing *E. coli* occurring in poultry birds of ages 13-17 weeks old (29%) (Table 1). This finding is not in agreement with other previous studies where the authors reported 44% prevalence of *E. coli* in poultry birds (Abdeltawabet al., 2015).

"The high occurrence of *E. coli* in poultry birds 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 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 types of samples, and for the fact that E. coli is a normal gut flora" (Shoaib et al., 2016, Salah-Eldinet al., 2015).

In this study, a total occurrence of 21% of ESBL-producing *E. coli* was observed in poultry birds. Higher prevalence (35.5%) of ESBL-producing *E. coli* in poultry birds was also reported in Maiduguri by Kwojiet al. This finding is lower than the findings of previous studies (Beninati et al., 2015, Stuart et al., 2012), where higher

occurrences of ESBL-producing E. coli were reported. It was also observed from this study that the highest occurrence of ESBL-producing *E. coli* was from broilers (21.2%) (Table 1). 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 pathogens in poultry birds with respect to type of poultry birds, it implies 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* is higher than findings of Shoaib et al. where 7.76% occurrence rate was reported. Furthermore, results of analysis of the occurrence of ESBL-producing *E. coli* in poultry birds based on age was statistically significant ($p>0.05$), and poultry birds with age range 13-17 weeks had the highest prevalence (29%). An analysis of housing system showed a higher prevalence in poultry birds raised using the Deep litter System (23.3%) but since no statistically significant difference was observed ($p>0.05$) between the Deep litter System and Battery cage System, it is implied that both systems are good conditions that support the growth of antimicrobial resistant pathogens. Antimicrobial susceptibility testing revealed interesting patterns with resistance rates observed in the majority of antimicrobial agents tested. These findings are similar to studies conducted by Mshanaet al., (2009). In this study, high resistance rates to beta-lactam drugs, namely cefotaxime (100%), ceftazidime (100%) were observed among the isolates investigated.

5. CONCLUSION

The study affirmed the presence of Extended Spectrum Beta-Lactamase producing E. coli in poultry birds from poultry farms in the study area. This is of serious public health significance since poultry birds are reared in close proximity to human population and may disseminate these resistant pathogens in the environment and in-contact to farm personnel. Poultry farm or meat products might be an important source of ESBL-producing *Escherichia coli* bacteria in Benin City leading to difficult to treat infections in humans.

6. RECOMMENDATIONS

1. Public enlightenment of poultry farmers on the consequence of antibiotics misuse should be done.
2. Routine detection and surveillance of ESBL-EC producers among poultry birds should be encouraged.
3. There should be public discouragement and strict regulation on over-the-counter sale of drugs to the public.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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