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Poultry slaughterhouse wastewater as reservoirs for spreading extended-spectrum beta-lactamase-producing *Escherichia coli* in Abakaliki, Nigeria

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Abstract. *Ugbo EN, Jacob JI, Effendi MH, Witaningrum AM, Agumah BN, Ugbo AI, Moses BI. 2023. Poultry slaughterhouse wastewater as reservoirs for spreading extended-spectrum beta-lactamase-producing* Escherichia coli *in Abakaliki, Nigeria. Biodiversitas 24: 4960-4966.* The emergence of antimicrobial and multi-resistance extended-spectrum beta-lactamase-producing organisms (ESBL) from the environment and hospitals is a serious global health concern. This research focused on the role of poultry slaughterhouse wastewater as a reservoir for spreading extended-spectrum beta-lactamase-producing *Escherichia coli*. Exactly 110 poultry slaughterhouses wastewater was collected using sterile universal containers from abattoirs in Abakaliki. The samples were analyzed microbiological for the presence of *E. coli* using standard techniques. The *E. coli* isolates found were subjected to a sensitivity test to antibiotics using the Kirby-Bauer disc diffusion method and further tested for ESBL-producing *E. coli* using a double disc synergy test and chromogenic media Brilliance ESBL agar. Of 110 wastewater samples analyzed, 55 (50.0%) were positive for *E. coli* contamination. The *E. coli* isolates showed antibiotic resistance ranging between 21.8% (amoxicillin clavulanic acid) to 69.1% (tetracycline), and good susceptibility was observed on cefepime (72.7%). Thus, among 55 *E. coli* isolates, 11(20.0%) were ESBL-producing *E. coli* with resistance patterns as follows: TET-STX-CAZ-CTX-FEP (four isolates); TET-STX-CAZ-CTX (four isolates); TET-STX-CAZ-CTX-FEP-CRO (three isolates), and average multidrug resistance index of 0.7. This study revealed that poultry slaughterhouse wastewater was a reservoir for multidrug resistance ESBL-producing *E. coli*. Therefore, properly treating wastewater from poultry production or farms before disposing into the drainage channel or water bodies is highly recommended to avoid spreading ESBL-producing organisms from animal waste products to humans and their environment.

Keywords: ESBL, *Escherichia coli*, human health, poultry wastewater

INTRODUCTION

In today's world, poultry meat is mostly consumed due to its high reproductive ability, nutritional value, relatively low sales prices, excellent space utilization, and feeding treatment. Production and consumption of poultry meat at this time have grown rapidly in almost every developed and developing country worldwide (Cooreman-Algoed et al. 2022). Food products made from chicken meat are globally popular among people and are considered a better choice for consumers because they can be quickly prepared and mixed with various foodstuffs. The modern days consumers in developed and developing countries are dependent on chicken meat products as their usual choice of meal due to their lifestyle (Kalakuntla et al. 2017). *Escherichia coli* is a major pathogen of wide interest in commercially farmed poultry, causing significant economic losses. *Escherichia coli*, on the other hand, is an excellent bacterium to be used as a model microorganism for detecting antimicrobial resistance (Benameur et al. 2019). *Escherichia coli* is an important extended-spectrum beta-lactamase (ESBL)-producing species because of its ability to shift its antimicrobial resistance (AMR) phenotypes in environments outside the host, colonize a wide variety of species, and evolve from a commensal, antimicrobial-susceptible bacterium into a resistant, pathogenic organism (Nakayama et al. 2022). It is often used as an indicator of the selective antimicrobial pressures on Enterobacteriaceae, is common among the intestinal tracts of warm-blooded hosts, and can serve as a reservoir for ESBL genes, actively participating in horizontal gene transfer to other bacteria, including pathogenic ones (Barrera et al. 2019). *Escherichia coli* can produce extended-spectrum beta-lactamase (ESBL), which is antibiotic-resistant (Wibisono et al. 2021). ESBL enzymes can hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Wibisono et al. 2021; Widodo et al. 2023). Exposure to large amounts of beta-lactam antibiotics can induce the production and mutation of beta-lactamase-type enzymes, including ESBL enzymes (Yanestria et al. 2022; Wibisono et al. 2020).

Bacteria from animals may carry clinically relevant resistance genes associated with resistance to veterinary and human antimicrobials (Ansharieta et al. 2021; Riwu et al. 2020; Tyasningsih et al. 2022). Exposure to multidrug-resistant bacteria during poultry production, slaughtering, and distribution can be a transmission vector to occupationally exposed workers (Kim et al. 2021; Widodo et al. 2023). *Escherichia coli* is a commensal and pathogenic bacterium among food-producing animals, and its health implications are of serious global concern. The most common ESBL gene types produced by *E. coli* include TEM (named Temoniera in Greece), SHV (Sulphydral variables), and CTX-M (a reference to its preferential hydrolytic activity against Cefotaxime, M for Munich). Studies have shown that ESBL genes previously found on chromosomes, now carried on plasmids are derivatives of plasmid-mediated β-lactamases such as *bla*TEM as well as types of environmental derivatives such as *bla*CTX-M (Aworh et al. 2020; Oduro-Mensah et al. 2016). Function presence of cellular genetic elements such as plasmids, insertion sequences, and transposons contributes to the plasticity of the *E. coli* genome (Savin et al. 2020). Treating these infections is one of the main challenges for humanity; multidrug-resistant *Escherichia coli* causes the majority of life-threatening bacterial infections in human and animal healthcare worldwide (Suetens et al. 2018). This threat requires serious attention to achieve higher productivity from man and his animals (Ieva et al. 2020). The use and misuse of drugs in poultry production, livestock animals, and human clinics accelerate the emergence of drug-resistant strains (Effendi et al. 2021). Pathogenic bacteria that produce extended-spectrum beta-lactamase (ESBL) enzymes, especially members of the Enterobacteriaceae family, such as *Escherichia coli*, have been reported in Nigeria (Ugbo et al. 2020).

The wastewater from poultry farms and abattoirs can be suspected as a source of antimicrobial-resistant bacteria with clinical implications and is important for their dissemination to the environment (Savin et al. 2020). Wastewater from poultry production has been suggested as reservoirs for spreading extended-spectrum beta-lactamase-producing *Escherichia coli.* Antimicrobial-resistant bacteria and partially metabolized antimicrobials carried in wastewater from poultry slaughterhouses may end up in surface waters and cropland via the sewage system (Verburg et al. 2019). ESBL-producing *E. coli* has been isolated from poultry (Effendi et al. 2021), livestock (Putra et al. 2020), domestic chicken (Rahmahani et al. 2020), cloacal and boot swabs in farms (Gundran et al. 2019), wastewater and process water from slaughterhouses (Savin et al. 2020), and commercialized chicken carcasses (Cyoia et al. 2019). The food chain is also an indicator of the transmission of multidrug-resistant bacteria to humans and animals due to close contact (Wibisono et al. 2020). Multidrug-resistant ESBL-producing *E. coli* might accumulate in processing waters and wastewater during poultry production. These waters may represent potential reservoirs that can contribute to a broad spread of multidrug resistance to other environmental ecosystems, including surface waters. Information on the role of poultry production wastewater in disseminating multidrug-resistant ESBL-producing *E. coli* is scarce in Abakaliki, Nigeria, and needs to be studied. Thus, this research focused on the role of poultry slaughterhouses wastewater as a reservoir for spreading extended-spectrum beta-lactamase-producing *Escherichia coli.*

MATERIALS AND METHODS

Study design and sample collection

A total of one hundred and ten (110) samples were collected from poultry slaughterhouses at Abakaliki in August 2022 in Ebonyi State, Nigeria. The samples were collected from selected poultry slaughterhouses directly from the outlet of wastewater discharging points. The wastewater was taken immediately before being discharged to the receiving water body/river. Exactly 50 mL were collected in a sterile plastic container (Carl Roth GmbH, Karlsruhe, Germany) and transported in cool boxes to the laboratory in the Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki Nigeria, for analysis. Samples were stored at 4°C before analysis, and each poultry slaughterhouse was sampled.

Isolation and identification of bacteria

At the time of laboratory analysis, 50 mL of wastewater samples were pre-filtered using sterilized gauze to remove the macro-particles and fine particles. After that, each sample was filtered again using the EZ-Fit filtration system with 0.45 µm pore size filter membranes (Merck Millipore, Darmstadt, Germany). After filtration, the filter membranes were transferred to 10 mL TSB (Carl Roth GmbH, Karlsruhe, Germany) containing 2 µg/mL cefotaxime (VWR International, Darmstadt, Germany) followed by overnight incubation at 37°C and shaking at 200 rpm. The overnight cultures were diluted up to 1,000-fold dilution. For each sample, 10 µL of the dilutions were inoculated on eosin methylene blue agar (EMBA) and chromogenic media Brilliant ESBL agar plates (Thermo Scientific, USA) and incubated overnight at 37°C. ESBL-producing colonies were sub-cultured to achieve pure cultures. Pure colonies were selected and stored for further verification and characterization/identification using Gram staining and biochemical tests, IMVIC (Indol-motility, methyl red, Voges Proskauer, citrate) and TSIA (Triple sugar iron agar) (Homeier-Bachmann et al. 2021; Widodo et al. 2020).

Antimicrobial Susceptibility Test (AST)

Antimicrobial susceptibility test (AST) was carried out on suspected ESBL-producing *E. coli* from chromogenic media Brilliance ESBL agar plates (Thermo Scientific, USA) using cephalosporin antibiotics, which includes, (ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), ceftriaxone (CRO; 30 µg), sulfamethoxazole-trimethoprim (STX; 25 µg), tetracycline (TET; 30 µg), cefepime (FEP; 30µg) and amoxicillin-clavulanic acid (AMC; 30 µg) (Oxoid Ltd)). Mueller Hinton agar (MHA) was prepared according to the manufacturer's instructions. Colonies of *E. coli* from EMBA were picked with a sterile wire loop, inoculated into a tube containing 5 mL of peptone water, and adjusted to match 0.5 MacFarland equivalents. Sterile swabs were collected and inoculated isolates onto the Mueller Hinton agar. The antibiotic discs were placed 30 mm away from each other and 15 mm from the edge of the plates and allowed to diffuse for about 10 minutes. The plates containing the *E. coli* isolates were incubated at 37°C for 18-24 hours under aerobic conditions. The diameter of the inhibition zone produced by the isolates was measured, recorded, and interpreted according to Clinical Laboratory Science Institute (CLSI 2020).

ESBL confirmation using Double Disc Synergy Test (DDST)

Moreover, using a double disc synergy test, the *E. coli* isolates resistant to the cephalosporin class were further investigated for extended-spectrum beta-lactamase. This was performed on a Muller Hinton agar with 3 major antibiotics to detect extended-spectrum beta-lactamase-producing organisms. The antibiotics used were ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg, and amoxicillin-clavulanic acid (AMC) 30 µg, which were placed in a parallel form at a distance of 15 mm from each other with amoxicillin clavulanic acid being the center disc. The culture plates containing the suspected ESBL-producing *E. coli* were impregnated with the earlier disc and incubated at 37°C for 18-24 hrs. The isolates were considered to be ESBL producers when there was a synergy between the center disc and the two discs with an increase in zone diameter of ≥5 mm for any of the antibiotics tested in combination with the clavulanic acid, but when tested alone, it was confirmed as ESBL producer (Effendi et al. 2021; Yanestria et al. 2022).

RESULTS AND DISCUSSION

The prevalence of *E. coli* isolates from poultry slaughterhouse wastewater samples was 55/110 (50.0%), as shown in Table 1. The resistance profiles of 55 isolates were as follows: ceftazidime (34.5%), cefotaxime (61.8 %), ceftriaxone (47.3%), sulfamethoxazole-trimethoprim (65.5%), tetracycline (69.1%), cefepime (27.3%) and amoxicillin-clavulanic acid (21.8). However, *E. coli* isolates showed higher susceptibility to 4th-generation cephalosporins (cefepime) and amoxicillin-clavulanic acid with 72.7% and 78.2% susceptibility frequencies, respectively (Table 2). The pattern of antibiotic resistance of *E. coli* isolates from slaughterhouse wastewater is shown in Figure 1. However, out of 55 *E. coli* isolates studied, 11 (20.0%) were ESBL-producing *E. coli* (Table 3). ESBL-producing *E. coli* produced green colonies on chromogenic Brilliance ESBL agar, while ESBL-negative *E. coli* produced colorless colonies (Figure 2). The synergistic effect of ceftazidime-amoxicillin/clavulanic acid-cefotaxime showed ESBL-producing *E. coli*, as presented in Figure 3. The ESBL-producing *E. coli* isolates had different verities of resistance pattern phenotype including, TET-STX-CAZ-CTX-FEP (four isolates), TET-STX-CAZ-CTX (four isolates), and TET-STX-CAZ-CTX-FEP-CRO (three isolates). The ESBL-producing *E. coli* isolates presented resistance index ranging from 0.6 to 9.0, which were very high. The total average multidrug resistance index of 0.7 was observed after summing the resistance index of all the isolates to the tested antibiotics (Table 4).

Table 1. Prevalence of *Escherichia coli* isolates in poultry wastewater samples from slaughterhouses

Sample source	Sample size	Number of positive samples for E. coli	Percentage (%)
Poultry wastewater	110	55	50.0

Table 2. Antibiogram of *Escherichia coli* isolates in poultry wastewater samples from slaughterhouses

Antibiotics	Number of *E. coli* in percentage	
	Susceptible	Resistant
Cefepime	40 (72.7)	15 (27.3)
Ceftazidime	36 (65.5)	19 (34.5)
Ceftriaxone	29 (52.7)	26 (47.3)
Tetracycline	17 (30.9)	38 (69.1)
Sulfamethoxazole	19 (34.5)	36 (65.5)
Cefotaxime	21 (38.2)	34 (61.8)
Amoxycillin/clavulanic acid	43 (78.2)	12 (21.8)

Table 3. Prevalence of ESBL-producing *Escherichia coli* isolates in poultry wastewater samples from slaughterhouses

Sample source	Sample size	Number of positive samples for *E. coli* (%)	Positive for ESBL (%)
Poultry wastewater	110	55 (50.0)	11 (20.0)

Table 4. Antibiotic resistance pattern and index of ESBL-producing *Escherichia coli* isolates

List of isolates	Antibiotic resistance pattern	Resistance index
*E. coli*A	TET-STX-CAZ-CTX-FEP	0.7
*E. coli*B	TET-STX-CAZ-CTX	0.6
*E. coli*C	TET-STX-CAZ-CTX	0.6
*E. coli*D	TET-STX-CAZ-CTX-FEP	0.7
*E. coli*E	TET-STX-CAZ-CTX-FEP-CRO	0.9
*E. coli*F	TET-STX-CAZ-CTX	0.6
*E. coli*G	TET-STX-CAZ-CTX-FEP	0.7
*E. coli*H	TET-STX-CAZ-CTX	0.6
*E. coli*I	TET-STX-CAZ-CTX-FEP-CRO	0.9
*E. coli*J	TET-STX-CAZ-CTX-FEP	0.7
*E. coli*K	TET-STX-CAZ-CTX-FEP-CRO	0.9
Total no. of isolates (11)		7.9/11 (0.7)

Note: Ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), tetracycline (TET), sulfamethoxazole-trimethoprim (STX), and cefepime (FEP); Average resistance index: 0.7

Discussion

The practice of discharging wastewater from poultry slaughterhouses into the environment/water bodies through drainage channels such as gutters and other water channels is commonly practiced in Nigeria by the abattoir owner/users. Thus, this has contributed greatly to disseminating antibiotic resistance organisms such as ESBL-producers in the environment. The present results showed that out of 110 analyzed wastewater samples, 55 (50%) were positive for *E. coli* contamination from poultry meat processing wastewater. This observation agrees with the findings of Mabel et al. (2020), who reported a prevalence value of between 40% to 54%. However, Iliya et al. (2019) reported a prevalence rate of *E. coli* to be 60% in Maiduguri, Nigeria. *Escherichia coli* isolates are also detected in 74.6% of the chicken carcasses (Telli et al. 2022). A similar study conducted in India reported a prevalence rate of 28% (Hussain et al. 2019), although this is a lower rate than the present study's findings. Enterobacteriaceae, such as *E. coli,* are zoonotic bacteria that can cause human diseases and can be present at high levels in abattoir wastewater (Veenemans et al. 2018). Falgenhauer et al. (2019) examined abattoir waste for bacteria with potential risk to human health at an abattoir in Nigeria; they reported *E. coli* O157: H7 and other Enterobacteriaceae from slaughterhouse wastewater. *Escherichia coli* is distributed among poultry of all ages, and its natural inhabitant is the gut of poultry birds and most other animals. Normally, it is kept in check by another bacterium in the gut, but if present in large colonies, it can cause severe discomfort, illness, and mortality. *Escherichia coli* is opportunistic in nature and grows rapidly in times of stress on poultry, such as transportation to the markets. This organism can find its way to the water bodies during processing in slaughterhouses since the wastewater is discharged directly to the river from the abattoir via drainage systems (Adebowale et al. 2016).

Several African studies have found *E. coli* and *Salmonella* species in slaughterhouse wastewater and effluent water from abattoir treatment facilities (Oloso et al. 2018; Gupta et al. 2021). It has been suggested that the presence of *E. coli* and other Enterobacteriaceae, which can be multidrug resistant in slaughter wastewater, is associated with the dumping of abattoir waste into water bodies (Saleem et al. 2017). As reported by researchers, *E. coli* is one of the major predominant organisms associated with poultry wastewater, accounting for 22% and above. Similar findings were reported in previous studies by Adebowale et al. (2016), where they reported *E. coli* as the most predominant bacterial organism identified in their study to be associated with wastewater. Another study by Oluwasile et al. (2014) showed that *E. coli* is the most common organism associated with bacteriuria, especially among the slaughters. Agyare et al. (2019) reported the presence of *E. coli* from wastewater in Jos, Nigeria. Aworh et al. (2020) also found these bacteria among poultry slaughter workers in Abuja, Nigeria.

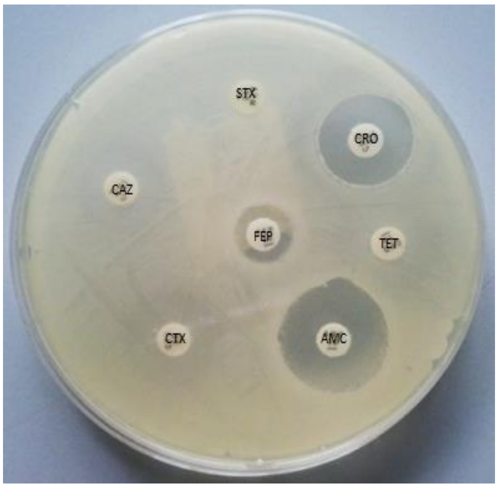

Figure 1. Antibiotic resistance pattern of *Escherichia coli* isolates from poultry slaughter wastewater. Note: Ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), tetracycline (TET), sulfamethoxazole-trimethoprim (STX), amoxicillin clavulanic acid (AMC), and cefepime (FEP)

Figure 2. ESBL-producing *Escherichia coli* produced green colonies on chromogenic Brilliant ESBL agar

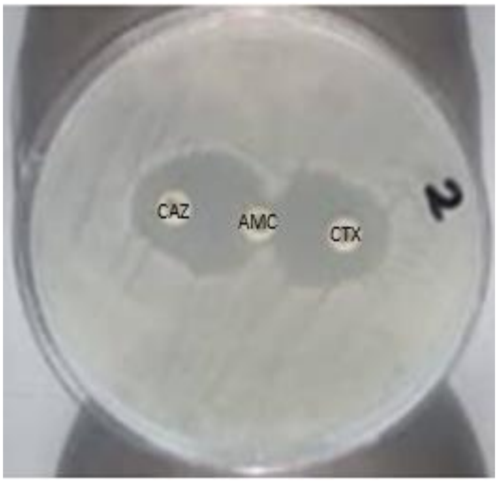

Figure 3. DDST for confirmation of ESBL-producing *Escherichia coli* isolated from poultry slaughterhouse wastewater. Note: Ceftazidime (CAZ), cefotaxime (CTX), and amoxicillin clavulanic acid (AMC)

Pathogens such as antimicrobial resistance *E. coli* can spread from animal to man in different ways; for example, via direct contact, consumption of food or water that is contaminated, indirect contact via objects that are contaminated, and transmission by vectors and by aerosols (Zankari et al. 2016; Nguyen et al. 2019). A study by Oduro-Mensah et al. (2016) suggested that animals can transfer pathogens to humans and other animals from abattoir waste by feeding on the same, and water contaminated with pathogens also causes infection in animals and humans drinking the water or eating crops or foods contaminated by the water.

The antibiogram of *E. coli* isolated from wastewater showed that the isolates had the highest susceptibility to amoxicillin-clavulanic acid (78.2%), followed by cefepime (72.7%) and the least susceptibility was recorded on tetracycline (30.9%). A similar study also revealed that *E. coli* isolates were highly sensitive to amoxicillin-clavulanic acid (Saleem et al. 2017), similar to this study's current findings. Notably, amoxicillin-clavulanic acid and cefepime antibiotics were effective against most of the bacteria isolates studied. This result indicates that the antibiotic sensitivity pattern of isolates associated with wastewater from poultry meat processing sites may vary significantly among different locations compared to the other researcher's findings. The present study revealed that *E. coli* isolates showed high resistance to tetracycline (69.1%) and sulfamethoxazole-trimethoprim (65.5%). This agrees with the observation of (Yanestria et al. 2022), who reported that *E. coli* from poultry wastewater is 56.25% resistant to sulfamethoxazole-trimethoprim. Meki et al. (2020) also reported the resistance profile of *E. coli* to trimethoprim/sulfamethoxazole (73.79%) and tetracycline (86.89%). Savin et al. 2021 observed *E. coli* from poultry slaughterhouse wastewater to show antibiotic resistance to cefotaxime and ceftazidime, with resistance percentages of 51.4 and 58, respectively. However, *E. coli* isolates were found to show varied resistance to the antibiotics tested. Mabel et al. (2020) reported that chickens are known as a reservoir for the spread of antimicrobial-resistant organisms such as *E. coli* to humans and the environment globally. In Nigeria, antimicrobial drugs are easily accessible for poultry production, preventive, and therapeutic purposes, although many poultry farm owners abuse antibiotics.

Out of 55 (50.0%) of *E. coli* isolated, 11 (20.0%) were ESBL-producing *E. coli*. The resistance pattern showed by the ESBL-producing *E. coli* phenotypically included TET-STX-CAZ-CTX-FEP (four isolates), TET-STX-CAZ-CTX (four isolates), and TET-STX-CAZ-CTX-FEP-CRO (three isolates), with an average multidrug resistance index of 0.7. The prevalence of ESBL-producing *E. coli* in poultry slaughterhouse wastewater was 39.4%. The prevalence of ESBL-producing *E. coli* was reported at 37.8% from chickens by Aworh et al. (2020), which is closely in line with the observation of this study. Iliya et al. (2019) observed that the occurrence of *E. coli* was 67.6%, and 32.0% were ESBL-producing *E. coli*. Research from Rio de Janeiro, Brazil, reported that the most prevalent multi-resistant extended-spectrum beta-lactamase (ESBL) producing isolates from hospital wastewater were *E. coli*, *Klebsiella pneumoniae,* and *Enterobacter cloacae* (Mabel et al. 2020). However, wastewater was identified as a potential exposure source of ESBL-producing *E. coli* (Gomi et al. 2017). Even though *E. coli* is generally considered a relatively harmless inhabitant of the human and animal gut; major public health risks may be associated with the spread of ESBL-producing bacteria. Colonization of ESBL-producing bacteria may disseminate and transfer ESBL-encoding genes to other pathogens through horizontal gene transfer with mobile elements such as bacteriophages, plasmids, and transposons (Iliya et al. 2019). Although it is relatively harmless for healthy individuals, these opportunistic bacteria may cause disease in more vulnerable individuals, such as hospitalized individuals, the elderly, or newborns. Exposure to ESBL-producing pathogenic *E. coli* variants may directly result in hard-to-treat infection and healthy individuals.

In conclusion, the present study identified *E. coli* from poultry slaughterhouse wastewater with prevalence of 50.0% and ESBL-producing *E. coli* at 20.0%. Thus, poultry slaughterhouse wastewater is important in the reservoirs of ESBL-producing organisms that colonize the environment. The presence of pathogenic bacteria such as ESBL-producing *E. coli* in poultry slaughterhouse wastewater that is finally discharged into the environment and receiving water bodies in Abakaliki, Ebonyi State, Nigeria, can pose serious public health risks to the human populace. These organisms can find their way back to human and their environs through food chains. Thus, there is a need for poultry farm and slaughterhouse owners to be educated on proper hygiene practices and the importance of proper treatment of poultry effluents and wastewater before discharging into the water bodies. Improper usage of antibiotics by poultry farm owners should be discouraged, and they are highly recommended always to seek the advice of veterinarians when caring for and treating their birds.

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