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Characterization of multidrug-resistant extended spectrum β-lactamase producing *Escherichia coli* from commercial swine farms in Ohaukwu local government Area, Ebonyi State Nigeria

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Abstract

Background: The expanding use of antimicrobials in livestock is an important contributor to the worldwide rapid increase in antimicrobial resistance (AMR). The transfer of antibiotic resistance to humans, which usually occurs via the food chain, is of concern for human health. This study assessed the incidence of multiple antibiotic resistance of Extended Spectrum β-lactamase producing *Escherichia coli* isolated from swine in Ohaukwu local government area of Ebonyi State, Nigeria.

Methodology: A total of 400 rectal and nasal swabs samples were randomly collected from four pig farms and analyzed for the presence of ESBL-producing *E. coli*. Phenotypic detection of ESBL-producing *E. coli* was done using double disk synergy test (DDST). Antibiotics susceptibility testing of ESBL-producing *E. coli* was determined against different classes of antibiotics using Kirby–Bauer disk diffusion method and multiple antibiotic resistance index (MARI) was determined.

Results: Out of 400 swine samples collected, sow/piglets have 164(41.0%), the weaners 140(35.0%) while the finishers were 96 representing 24%. Furthermore, it revealed that out of the 400 samples studied, 157 (39.3%) were *E. coli* positive, rectal had 85 (42.5%) and nasal had 72 (36.0%). Exactly 19 (12.1%) were ESBL-producing *E. coli* out of the 157 isolates analyzed, 13 (15.3%); 6 (8.3%) were from rectal and nasal swabs respectively. The ESBL-producing *E. coli* from swine samples showed varying range of resistance to the antibiotic tested. The ESBL-producing *E. coli* isolates from rectal swab showed high resistant profile to Amoxicillin/Clavulanic acid, (76.9%); Cefepime, (92.3%); Ceftazidime, (84.6%); Nalidixic acid, (92.3%); and Piperacillin/Tazobactam and Cefoxitin, (100.0%). The ESBL-producing *E. coli* isolates from nasal swabs were (100.0%) resistance to Amoxicillin/Clavulanic acid, Cefepime, Cefoxitin, Colistin, Nalidixic acid and were (100.0%) susceptible to meropenem. The multi antibiotic resistance index of the isolates ranged from 0.33 to 0.83 with average index of 0.66.

Conclusion: The ESBL-producing *E. coli* isolates from swine was at high prevalence. This therefore is a big threat to public health and calls for a strict measure in the choice of antibiotics used in swine productions

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Keywords: ESBL; *E. Coli*; Multi Antibiotic Resistance; Swine; Ohaukwu; Ebonyi

1. Introduction

The discovery and development of the antibiotic penicillin during the 1900s gave a certain hope to medical science, as the therapeutic paradigm changed with its introduction into clinical use [1] which have continued to save lives around the globe [2]. Created originally by microorganisms to protect themselves, microorganisms use antibiotics to exterminate surrounding neighbours, and allow them to colonize and dominate different habitats [3].

Antibiotic resistance in bacteria is generally a natural phenomenon for adaptation to antimicrobial agents. Once bacteria become resistant to some antibiotic, they pass on this characteristic to their progeny through horizontal or vertical transfer. Increased dissemination of antibiotic resistance bacteria and antibiotic resistance genes in the environment may result from selective pressures imposed by human activities. The indiscriminate and irrational use of antibiotics these days has led to the evolution of new resistant strains of bacteria that are somewhat more lethal compared to the parent strain. These activities include overuse of antibiotics in clinics, hospitals, and nursing homes [3-4]. Other activities include antibiotics use to prevent diseases and promote growth in farm animals and aquaculture [4-5]. All these singly and combined make it difficult to treat bacterial infection due to bacteria's ability to develop resistance against antimicrobial agents [6].

A number of microorganisms has been implicated to be mainly involved in the resistance process. Often called the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and enterobacteriaceae*), they are known for their capacity to "escape" from common antibacterial treatments and remain infective and invasive within and outside their host cells [7-8].

The domestic pig (*Susscrofa domesticus*) is one of the most important human food-producing animals, as over 40% of all meat consumed on earth comes from swine [9]. Antimicrobial agents, especially antibacterial agents, are used throughout the world, across a diverse array of extensive and intensive livestock production systems, to protect the health and welfare of livestock and to improve their performance. In recent years, antibiotics has found immense use in the animal husbandry both as anti-infective agents and as growth enhancers of some sort [10]. The use of antibiotics in the food production chain is usually seen as important in continuing a consistent supply of healthy and substantial animals, leading to greater profitability and efficient production [11,12,13]. This application of mass medication, and the use of broad- spectrum antibiotics in animal husbandry is common, because it is often impractical to treat animals individually [13].

Many bacterial diseases of livestock cause devastating losses of animal life and productivity. As a result, their keepers can lose their livelihoods and see a dramatic reduction in income, so there is often a great sense of urgency to treat affected animals early. Today, the agricultural industry relies on the use of antimicrobials to improve animal health and productivity, especially in intensively reared species [14,15]. However, there are a large number of bacterial pathogens that cause disease and it is frequently difficult to reach a conclusive diagnosis prior to instituting treatment [16]. This exploitation of antibiotics at low concentration over extended time favours the emergence of Antibiotic resistance strains.

Consequently, and worryingly, many antimicrobials that are regarded as important to human health are used in animal food production, such as Tetracyclines, Penicillins, and Sulphonamides [16,17]. The misuse, overuse and inappropriate use of these antimicrobials in animal husbandry has created enormous selective pressure, which increases and accelerates the likelihood that bacteria will adapt and multiply to produce a more resistant population. Thus, the farming industry has become an Antibiotic Resistance hotspot which spills down to the consumers directly or indirectly as studies have shown that the use of antibiotics in food-production animals, especially for non-therapeutic use, is linked to resistance in people who live on and near farms and even the general population via the food chain [18]. Primarily, the direct transmission of these antibiotic resistant bacteria to man is mainly via contact with companion animals and wildlife [12], as well as occupational exposure [19], resulting in mild to serious infections and even death [20, 21]. Indirect transfer routes include contamination of the soil, rivers and streams by farm effluents and manure spreading which makes its way to man. There are various stages and interactions in the food production chain at which antibiotic resistant bacteria can enter. First, in the physical environment, such as soil, air and water, resistant organisms can spread from animals. The consumption of water that is contaminated because of resistant bacteria from animal waste that is used as fertilizer is also another risk factor [17]. The application of antibiotics in various situations, from agriculture to the food industry, has resulted in a constant release of low-level antibiotic concentration into the water and soil through wastewater treatment plant effluents, sewage, agricultural waste and the application of manure fertilizer to fields and gardens, among others which all pose significant health implication [22].

The incidence of infections caused by extended–spectrum beta-lactamase producing resistant *E. coli* has risen unprecedentedly because they are becoming more resistant to multiple clinically important antibiotics [23, 24, 25]. Recently, peng *et al.,* [9] reported that the analysis of *E. coli* isolates from swine and other animals by antimicrobial susceptibility testing (AST) in combination with whole-genome sequencing (WGS) has facilitated a better understanding of Antimicrobial resistance development and dissemination. Due to the paucity of information on ESBL-producing *E. coli* in Ohaukwu Local Government Area, Ebonyi State Nigeria, it's therefore, pertinent to investigate the multiple antibiotic resistance pattern of the extended spectrum beta lactamase producing *Escherichia coli* isolated from Rectal and Nasal Swabs of Swine. This study was therefore undertaken to augment the currently available information and with a view to bridging the knowledge gap.

2. Materials and methods

2.1. Study area

This study was carried out in Ohaukwu Local Government Area of Ebonyi State. Ohaukwu Local Government Area has an estimated population of 196,000 (NPC 2006) with three major clans namely; Ezzangbo, Ngbo and Effium and has two constituencies namely; Ohaukwu-North Constituency and Ohaukwu-South Constituency. Ohaukwu covers an estimated area of 252 km^2. The area lies within latitudes 6^0 3' N to 6^0 50' N and longitudes 7^0 80' E to 8^0 00' E with climatic conditions such as rainy season (March-October) and dry season (October-February). Two distinct vegetative regions exist in the study area: the Savannah in the Northern part of the study area, and tropical rainforests in the southern parts. The selected clans in Ohaukwu were picked on the basis of their dense populations and more than 70% of the inhabitants engage in economic activities such as petty trading, swine farming, subsistent agriculture, hunting and fishing.

2.2. Sample Collection

A total of four hundred (400) swab samples of swine were collected from four different farms for the purpose of this study. Two hundred (200) rectal and nasal swabs each were collected from four different swine farms (50 rectal and 50 nasal swabs from farm (A, B, C and D) in Ohaukwu Local Government Area of Ebonyi State. The rectal and nasal swab samples were collected using sterile swab stick and all the samples were labeled with the unique sample number and date from different pens in each of the following sections: sows/piglets (164), weaners (140) and finishers (96) and the samples were transported to the laboratory at the Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, for routine microbiological analysis. Then samples were cultured on nutrient broth for isolation of *E. coli.*

2.3. Isolation, Purification and Characterization of Isolates

All the swine samples of rectal and nasal swabs were analyzed for the presence of *E. coli* by inoculating a loopful of each swine sample into a separate test tube of sterile nutrient broth and incubated at 37 °C for 24 h. After overnight incubation, a loopful of the turbid broth culture was aseptically seeded by streaking on sterile solidified MacConkey agar and incubated at 37 °C for 24h. Suspected *Escherichia coli* from positive cultures were identified by their characteristic appearance (color, consistency, shape) on the media. Each mucoid-pink colony was sub-cultured on sterilized solidified nutrient agar and incubated at 37 °C for 24 h. Further identification of *Escherichia coli* was based on standard microbiological technique which includes; Gram-staining, indole test, Citrate utilization test, oxidase test, carbohydrate fermentation test, motility test (hanging drop method), triple sugar iron test, methyl red (MR) test and colony morphology [26].

2.4. Antibiotic susceptibility testing

The antibiotic susceptibility patterns of the identified isolates were determined by disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. A 0.5 McFarland's equivalent standard of the test isolates each was inoculated on the surface of Mueller-Hinton agar (Merck; 105,437) plates using sterile swab stick. Antibiotic discs of Amikacin (15 µg), Amoxicillin/Clavulanic acid (30 µg), Cefepime (10 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Colistin (10 µg), Meropenem (10 µg), Nalidixic acid (30 µg), Ofloxacin (5 µg) and Piperacillin/Tazobactam (85 µg) (Oxoid, UK CT0264B) were placed 30 mm way from each other on the surface of the inoculated agar plates using sterile forceps. The antibiotics were allowed to diffuse for about 10minutes and were incubated at 37^0C for 18-24 hours as described by [27]. The diameters of inhibition zones were measured in millimeter (mm) with rule, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines [28].

2.5. Detection of extended spectrum beta-lactamase producing *Escherichia coli*

Escherichia coli isolated in this study were screened for beta-lactamase production. Phenotypical detection of ESBL-producing *E. coli* was carried out in only the bacteria isolates that showed reduced susceptibility to the 2nd and 3rd generation cephalosporins (such as Cefotaxime and Ceftriaxone) using the double disk synergy test (DDST) technique [29]. Standardized inoculums of the isolate (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on the Mueller-Hinton agar plates; and Amoxycillin/Clavulanic acid disc (20/10 µg) was placed at the center of the plate while cefotaxime (30 µg) and ceftazidime (30 µg) discs each was placed at adjacently distance of 15 mm away from the amoxycillin/clavulanic acid disc. The plates were incubated at 37°C for 18 - 24hours; and ESBL production was phenotypically inferred by expansion of the zone of inhibition of either Cephalosporin in the presence of Amoxicillin/Clavulanic acid than in its absence giving a dumb-bell shape.

3. Results

Table 1 showed the morphological features and biochemical characteristics of Escherichia coli isolated from swine samples in Ohaukwu local government area of Ebonyi State. The isolates were found to show greenish colouration on EMB. It was a Gram-negative rod after staining, with morphological and biochemical characterization showing the bacteria isolate to be Escherichia coli. The E. coli isolates from swine samples were also observed to produce acid/gas in TSI, oxidase, API, xylose, citrate, fructose negative and motility, red, indole, glucose, lactose, galactose, arabinose and sorbitol positive.

Table 1 Morphological and Biochemical characteristics of *Escherichia coli* from swine samples in Ohaukwu Local Government Area

Tests	**Biochemical**
Gram staining	-
Shape	Rod
Colour	Green-Sheen on EMB
TSI	A/G
Oxidase	-
Motility	+
API	-
Methyl red	+
Indole	+
Citrate	-
Glucose	+
Lactose	+
Galactose	+
Arabinose	+
Sorbitol	+
Xylose	-
Fructose	-
Probable/Suspected bacteria	*E. coli*

Keys: Positive, (-)- Negative,

3.1. Distribution of *Escherichia coli* from swine

In this research, a total of 400 swine samples (100 each from farm A, B, C, D) were analyzed for the presence of *Escherichia coli*. Overall occurrence rate of *Escherichia coli* for 157(39.3%) consisting of high proportion from farm D

51(51.0%) followed by farm C 49(49.0%) while the least occurrence rate was observed against farm A 29(29.0%) and farm B 28 (28.0%) as presented in figure 1 below.

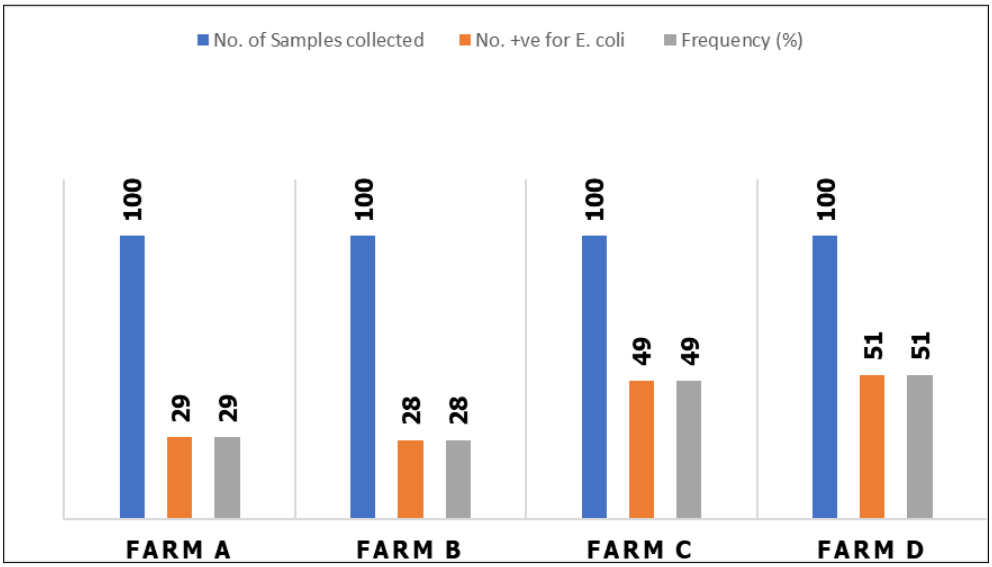

Figure 1 Distribution of *Escherichia coli* from swine samples in Ohaukwu Local Government area

3.2. Frequency of isolation of *Escherichia coli* from rectal and nasal swab

The frequency of *Escherichia coli* in different farms isolated from swine was highly prevalent in rectal swabs 85(54.1%) while nasal swabs had 72(45.9%). The distribution of *Escherichia coli* from rectal and nasal swab samples in swine in Ohaukwu Local Government Area differ significantly at P≥0.05. Thus, 18(62.1%); 14(50.0%); 29(59.2%) and 24(47.1%) of the *E. coli* isolates were from rectal swab collected from farm (A, B, C and D) respectively. The nasal swab harbored 11 (37.9%); 14(50.0%); 20(40.8%) and 27(52.9%) of the *E. coli* isolates from farm (A, B, C and D) respectively as shown in (Table 1).

Table 2 Frequency of isolation of E. coli from rectal and nasal swab samples of swine in Ohaukwu Local Government area

Sample Source	No. +ve for *E. coli* (%)	Rectal swab (%)	Nasal swab (%)
Farm A	29	18 (62.1)	11 (37.9)
Farm B	28	14 (50)	14 (50)
Farm C	49	29 (59.2)	20 (40.8)
Farm D	51	24 (47.1)	27(52.9)
Chi-Square	12.000[a]		
P-Value	0.213		
Total	**157**	**85 (54.1)**	**72 (45.9)**

3.3. Frequency of isolation of ESBL-Producing *Escherichia coli* with respect to swine type

The frequency of isolation of ESBL-Producing *Escherichia coli* with respect to swine type was highly prevalence in sow/piglet 10(52.6%) followed by weaners 7(36.8%) while finishers 2(10.5%) had least occurrence as shown in figure 2 below.

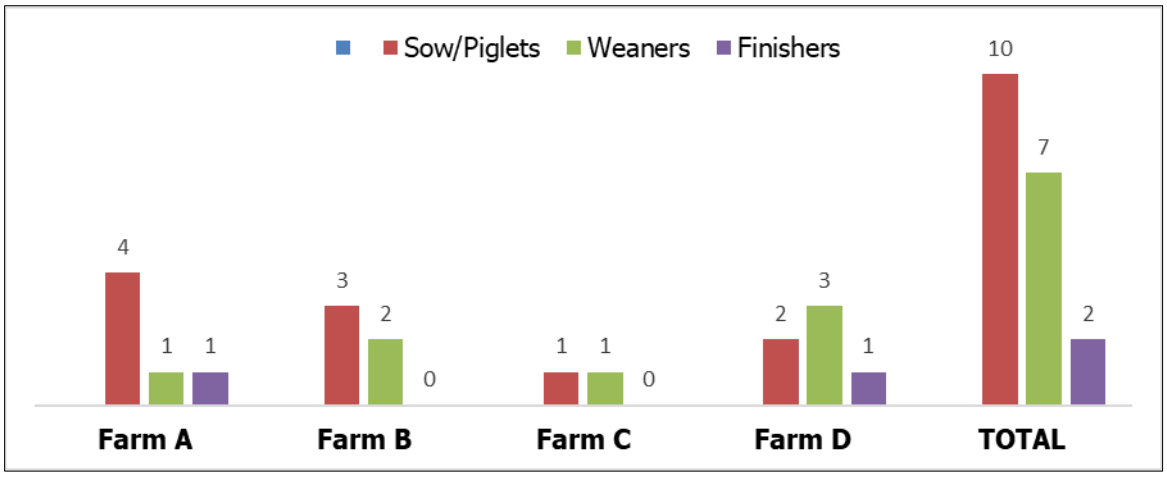

Figure 2 Frequency of isolation of ESBL-Producing *Escherichia coli* with respect to swine type

3.4. Overall distribution of ESBL-producing *E. coli* from rectal swab

Out of the 200 rectal swab sample studied (50 each from fram A, B, C, D), 85(42.5%) were positive for *E. coli*, whereas 13(15.3%) were ESBL-producing *E. coli*. However, 18(36.0%); 14(28.0%); 29(58.0%) and 24(48.0%) were positive for *E. coli* isolate from Farm (A; B; C; and D) respectively. ESBL-producing *E. coli* were identified from farm A; 6(33.3%), B; 3(21.4%), C; 0(0.0%), D; 4(16.7%) respectively as shown in (Table 3).

Table 3 Distribution of ESBL-producing *E. coli* in rectal swab from swine in Ohaukwu Local Government Area

Farm type	No. of Samples	No. +ve for *E. coli* (%)	ESBL-positive (%)
Farm A	50	18 (36.0)	6(33.3)
Farm B	50	14 (28.0)	3(21.4)
Farm C	50	29 (58.0)	0(0.0)
Farm D	50	24 (48.0)	4(16.7)
Total	200	85 (42.5)	13 (15.3)

3.5. Overall distribution of ESBL-producing *E. coli* from nasal swabs

Table 4 below shows that out of the 200 nasal swab samples analyzed (50 each from farm A, B, C, D), 72(36.0%) were positive for *E. coli*, and 6(8.3%) were ESBL-producing *E. coli*. Thus, 11 (22.0%); 14 (28.0%); 20 (40.0%) and 27 (54.0%) were positive for *E. coli* isolate from Farm (A; B; C; and D) respectively. The *E. coli* that produces ESBL was identified from the farms as follows: A; 0 (0.0%), B; 3 (21.4%), C; 1 (5.0%), D; 2 (7.4%) respectively as shown in (Table 4).

Table 4 Overall distribution of ESBL-producing *E. coli* from nasal swab from swine samples in Ohaukwu Local Government Area

Farm type	No. of Samples	No. +ve for *E. coli* (%)	ESBL-positive (%)
Farm A	50	11 (22.0)	0(0.0)
Farm B	50	14 (28.0)	3(21.4)
Farm C	50	20 (40.0)	1(5.0)
Farm D	50	27 (54.0)	2(7.4)
Total	**200**	**72 (36.0)**	**6 (8.3)**

3.6. Antibiotic susceptibility profile of *E. coli* from rectal swab samples

The antibiotic susceptibility profile of *Escherichia coli* from rectal swab samples of swine revealed that isolates from Farm A were highly resistant to amoxicillin/clavulanic acid100.0%, cefepime 100.0%, ceftazidime 100.0%, nalidixic acid 100.0% but were susceptible to meropenem 100.0%. Moreso, *E. coli* isolates from Farm B were also 100.0% resistant to amoxicillin/clavulanic acid, cefepime, ceftazidime and 100.0% susceptible to meropenem. The *E. coli* isolates from Farm C presented antibiotics resistance range from 13.8% to 100.0% and were all susceptible to meropenem. The Farm D isolates were 100.0% resistance to amoxicillin/Clavulanic acid, cefepime, cefoxitin and 100.0% susceptible to amikacin, meropenem and ofloxacin as shown in Table 5 below.

3.7. Antibiotic Susceptibility profile of *E. coli* from nasal swab samples

The antibiotic susceptibility profile of *E. coli* from nasal swab samples of swine shows that isolates from Farm A had antibiotics resistance range from 45.5% to 100.0% and all the isolates were susceptible to meropenem and cefepime. The *E. coli* isolates from nasal swab of swine in Farm B showed 100.0% resistant to Amoxicillin/Clavulanic acid, cefepime, ceftazidime and 100.0% susceptible to Meropenem. The isolates from Farm C had antibiotics resistance ranging from 25.0% to 100.0% and were all susceptible to meropenem. The Farm D isolates were 100.0% resistance to ceftriaxone, ceftazidime, colistin and cefepime as illustrated in Table 6 below.

3.8. Multiple Antibiotic Resistance Index (MARI) of *E. coli* isolates

Multiple antibiotic resistance index (MARI) of *E. coli* isolated from rectal swab and nasal swab were presented. The isolates with the code AR21, AR43, BR18, BR34, had similar antibiotics resistance index as CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA. Other isolates presented different antibiotics resistance indexes as show in the Table. The MARI index of the isolates ranged from 0.33 to 0.83 with average index of 0.66 as shown in (Table 7).

3.9. Antibiotic susceptibility Profile of ESBL producing *E. coli* isolates

The antibiotic profile of ESBL-producing *E. coli* from rectal swab and nasal swab of swine samples presented in Table 8 showed that significant differences ($P<0.05$) were observed in the susceptibility and resistance in the antibiotic tested against them. Highest resistant profile to ESBL-producing *E. coli* isolates from rectal swab was observed in Colistin (11.00 ± 1.00µg), Cefepime (10.33 ± 1.53 µg), Amoxicillin/Clavulanic acid (10.00 ± 1.00 µg), Piperacillin/Tazobactam (9.67 ± 1.53 µg), Nalidixic Acid (8.33 ± 1.53 µg), followed by Amikacin (5.33 ± 0.58 µg) while significant resistant decrease was recorded in Ofloxacin (52.00 ± 1.00 µg), Meropenem (2.00 ± 1.00 µg), Cefotaxime (4.00 ± 1.00 µg) and Cefoxitin (3.00 ± 1.00 µg). Meanwhile, the isolates were highly resistant to Meropenem (11.00 ± 1.00 µg), Ofloxacin (11.00 ± 1.00 µg), Ceftriaxone (9.00 ± 1.00 µg) and Cefoxitin (10.00 ± 1.00 µg). Compared to the rectal swab isolates, the ESBL-producing *E. coli* isolates from nasal swabs had the highest resistant profile to Amoxicillin/Clavulanic acid (5.00 ± 1.00 µg); Colistin, (5.00 ± 1.00 µg), Cefepime (4.00 ± 1.00 µg), Nalidixic Acid (4.00 ± 1.00 µg) and cefoxitin (3.00 ± 1.00 µg). Similarly, Cefotaxime (3.00 ± 1.00 µg), Ceftriaxone (3.00 ± 1.00 µg), Ofloxacin (5.00 ± 1.00 µg) and Amikacin (1.67 ± 1.53 µg) had the highest susceptibility profile against ESBL-producing *E. coli* isolated from nasal swabs (Table 8).

Table 5 Antibiotic susceptibility profile of *E. coli* from rectal swab samples of swine in Ohaukwu L.G.A

Sample source	**Farm A**		**Farm B**		**Farm C**		**Farm D**	
Antibiotics (µg)	***E. coli* (n=18)**		***E. coli* (n=14)**		***E. coli* (n=29)**		***E. coli* (n=24)**	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Amikacin (15)	2(11.1)	16(88.9)	0(0.0)	14(100)	4(13.8)	25(86.2)	0(0.0)	24(100)
Amoxicillin/Clavulanic acid (30)	18(100)	0(0.0)	14(100)	0(0.0)	29(100)	0(0.0)	24(100)	0(0.0)
Cefepime (10)	18(100)	0(0.0)	14(100)	0(0.0)	16(55.2)	13(44.8)	24(100)	0(0.0)
Cefotaxime	4(22.2)	14(77.8)	3(21.4)	11(78.6)	29(100)	0(0.0)	5(20.8)	19(79.2)
Cefoxitin (30)	17(94.4)	1(5.6)	10(71.4)	4(28.6)	27(93.1)	2(6.9)	24(100)	0(0.0)
Ceftriaxone (30)	4(22.2)	14(77.8)	5(35.7)	9(64.3)	23(79.3)	6(20.7)	21(87.5)	3(12.5)
Ceftazidime	18(100)	0(0.0)	14(100)	0(0.0)	25(86.2)	4(13.8)	22(91.7)	2(8.3)
Colistin (10)	17(94.4)	1(5.6)	12(85.7)	2(14.3)	27(93.1)	2(6.9)	9(37.5)	15(62.5)

Meropenem (10)	0(0.0)	18(100)	0(0.0)	14(100)	0(0.0)	29(100)	0(0.0)	24(100)
Nalidixic Acid (30)	18(100)	0(0.0)	13(92.9)	1(7.1)	29(100)	0(0.0)	23(95.8)	1(4.2)
Ofloxacin (5)	0(0.0)	18(100)	12(85.7)	2(14.3)	20(69)	9(31)	0(0.0)	24(100)
Piperacillin/Tazobactam (85)	15(83.3)	3(16.7)	14(100)	0(0.0)	29(100)	0(0.0)	16(66.7)	8(33.3)

Key: R-Resistance, S- Susceptible, n=Number of isolates, n_{ab} = Number of antibiotics used (12).

Table 6 Antibiotic susceptibility profile of *E. coli* from nasal swab samples of swine in Ohaukwu L.G.A

Sample source	Farm A		Farm B		Farm C		Farm D	
Antibiotics (µg)	*E. coli* (n=11)		*E. coli* (n=14)		*E. coli* (n=20)		*E. coli* (n=27)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin (15)	4(36.4)	7(63.6)	0(0.0)	14(100)	2(10)	18(90)	0(0.0)	27(100)
Amoxicillin/Culvanic acid (30)	11(100)	0(0.0)	14(100)	0(0.0)	20(100)	0(0.0)	21(77.8)	6(22.8)
Cefepime (10)	11(100)	0(0.0)	14(100)	0(0.0)	5(25)	15(75)	27(100)	0(0.0)
Cefotaxime	0(0.0)	11(100)	2(14.3)	12(85.7)	19(95)	1(5)	3(11.1)	24(88.9)
Cefoxitin (30)	11(100)	0(0.0)	13(92.9)	1(7.1)	18(90)	2(10)	25(92.6)	2(7.4)
Ceftriaxone (30)	9(81.8)	2(18.2)	2(14.3)	12(85.7)	15(75)	5(25)	27(100)	0(0.0)
Ceftazidime	7(63.6)	4(36.4)	14(100)	0(0.0)	16(80)	4(20)	27(100)	0(0.0)
Colistin (10)	11(100)	0(0.0)	10(71.4)	4(28.6)	17(85)	3(15)	27(100)	0(0.0)
Meropenem (10)	11(100.0)	0(0.0)	0(0.0)	14(100)	0(0.0)	20(100)	5(18.5)	22(81.5)
Nalidixic Acid (30)	5(45.5)	6(54.5)	13(92.9)	1(7.1)	20(100)	0(0.0)	20(74.1)	7(25.9)
Ofloxacin (5)	0(0.0)	11(100)	14(100)	0(0.0)	19(95)	1(5)	0(0.0)	27(100)
Piperacillin/ Tazobactam (85)	10(90.9)	1(9.1)	9(64.3)	5(35.7)	20(100)	0(0.0)	19(70.4)	8(29.6)

Source: Field work, 2023

Key: R-Resistance, S- Susceptible, n=Number of isolates, nab = Number of antibiotics used (12)

Table 7 Multiple Antibiotic Resistance Index (MARI) of *E. coli* isolated from rectal swab and nasal swab of swine in Ohaukwu LGA of Ebonyi State

ISOLATE SOURCE	ISOLATE CODE	ANTIBIOTIC CODE	NAB = 12	MARI INDEX
Farm A	AR15	CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.67
	AR21	CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.67
	AR27	CAZ-FEP-CT-CRO-AMC-FOX-NA		0.58
	AR43	CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.75
Farm B	BN10	CAZ-FEP-CRO-OFX-AMC-FOX-TPZ-NA		0.67
	BN14	CTX-CAZ-FEP-TPZ		0.33
	BN26	CTX-CAZ-FEP-CRO-AMC-TPZ-NA		0.58
	BR18	CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.75

	BR34	CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.75
Farm C	CN30	CTX-CAZ-FEP-CT-CRO-OFX-AMC-FOX-TPZ-NA		0.83
Farm D	DN45	CTX-CAZ-FEP-CRO-AK-AMC-FOX-TPZ-NA		0.75
	DR31	CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA		0.75
	DR40	CTX-CAZ-FEP-CT-CRO-AMC-FOX-NA		0.67
	DR45	CTX-CAZ-FEP-AMC-FOX-TPZ-NA		0.58
Total	(n=14)	AVERAGE MARI		0.66

Keys: AR = Farm A Rectal, BN = Farm B Nasal, BR = Farm B Rectal, CN = Farm C Nasal, DN = Farm D Nasal, and DR =Farm D Rectal swabs, NAB = Number of Antibiotics used (12).

Table 8 Antibiotic susceptibility and resistance profile of ESBL producing *E. coli* from swine samples of farms in Ohaukwu L.G.A

Sample type	**Rectal swab *E. coli* (n=13)**				**Nasal swab *E. coli* (n=6)**			
Antibiotics(µg)	Resistance (R)	R (%)	Susceptible	S (%)	Resistance (R)	R (%)	Susceptible	S (%)
Amikacin (15)	5.33 ± 0.58^{b}	38.5	7.67 ± 0.58^{de}	61.5	2.00 ± 1.00^{ab}	33.3	1.67 ± 1.53^{bc}	66.7
Amoxicillin/Culvanic acid (30)	10.00 ± 1.00^{cd}	76.9	6.00 ± 2.65^{cd}	23.1	5.00 ± 1.00^{c}	100	2.00 ± 1.00^{a}	0.0
Cefepime (10)	10.33 ± 1.53^{cd}	92.3	2.67 ± 1.53^{ab}	7.7	4.00 ± 1.00^{bc}	100	4.00 ± 1.00^{ab}	0.0
Cefotaxime (15)	4.00 ± 1.00^{ab}	30.8	9.00 ± 1.00^{ef}	69.2	2.00 ± 1.00^{ab}	16.7	3.00 ± 1.00^{bc}	83.3
Cefoxitin (30)	3.00 ± 1.00^{a}	100	10.00 ± 1.00^{ef}	0.0	3.00 ± 1.00^{bc}	100	2.33 ± 1.53^{abc}	0.0
Ceftriaxone (30)	4.00 ± 1.00^{ab}	23.1	9.00 ± 1.00^{ef}	76.9	3.67 ± 1.53^{bc}	16.7	3.00 ± 1.00^{ab}	83.3
Ceftazidime	10.00 ± 1.00^{cd}	84.6	3.00 ± 1.00^{ab}	15.4	3.00 ± 1.00^{bc}	66.7	1.00 ± 1.00^{abc}	33.3
Colistin (10)	11.00 ± 1.00^{d}	100	2.00 ± 1.00^{a}	0.0	5.00 ± 1.00^{c}	100	5.00 ± 1.00^{a}	0.0
Meropenem (10)	2.00 ± 1.00^{a}	0.0	11.00 ± 1.00^{f}	100	1.00 ± 1.00^{a}	0.0	2.00 ± 1.00^{c}	100
Nalidixic Acid (30)	8.33 ± 1.53^{c}	92.3	4.67 ± 1.53^{bc}	7.7	4.00 ± 1.00^{bc}	100	2.00 ± 1.00^{ab}	0.0
Ofloxacin (5)	2.00 ± 1.00^{a}	0.0	11.00 ± 1.00^{f}	100	1.00 ± 1.00^{a}	0.0	5.00 ± 1.00^{c}	100
Piperacillin/Tazobactam (85)	9.67 ± 1.53^{cd}	100	3.33 ± 1.53^{ab}	0.0	4.00 ± 1.00^{bc}	50	2.00 ± 1.00^{ab}	50
P-Value(α)	0.0000		0.0000		0.0000		0.001	

Key: R (%) –Resistance Percentage, S (%) – Susceptible Percentage, n=Number of isolates. Means along column with the different alphabets are significantly different at $P<0.05$.

4. Discussion

In nature, microorganisms are everywhere. They are present in almost every area of our surroundings. Pathogenic bacteria pose a significant threat to public health. When caregivers for animals come in contact with animals or animal wastes, as well as items that contain infectious organisms, during animal husbandry. The study yielded an overall occurrence rate of 39.3% for *Escherichia coli*, of which the highest proportion came from farm D 51 (51.0%), followed by farm C 49 (49.0%), and the lowest percentage was found against farms A 29 (29.00%) and B 28 (28.0%), as shown in figure 1. This confirms the reports of Li *et al*., [30] and Ajayi *et al.,* [31]. In several swine farms, the prevalence of

Escherichia coli was found to be 85 (54.1%) and 72 (45.9%) in rectal swabs and nasal swabs respectively. Table 1 displays a significant difference (P≥0.05) in the distribution of *Escherichia coli* in rectal and nasal swab samples from swine in Ohaukwu Local Government Area.

Globally, the frequency of *E. coli* isolates that produce ESBLs in food animals has been rising. The extended spectrum beta lactamase-producing *E. coli* was effectively isolated in this investigation from nasal and rectal swabs of swine in Ohaukwu LGA, Ebonyi state. According to figure 1 above, the investigation found that the total occurrence rate of *Escherichia coli* for 157 was 39.3%. This included a large proportion from farm D 51 (51.0%) and farm C 49 (49.00%), while the lowest occurrence rates were seen against farms A 29 (29.00%) and B 28 (28.0%). Regarding the swine type, the frequency of isolation of ESBL-producing *Escherichia coli* was highest in sow/piglets (10.6%), followed by weaners (7.8%), and finishers (20.5%), with the lowest frequency as previously indicated. The ambient pollutants, improper sanitation surrounding the animal home, and exposure to high doses of antibiotics as growth promoters were the causes of the presence of beta lactamase generating *E. coli* in the samples. This finding is consistent with that of [32] who found that *Escherichia coli* isolated from animals that produce food was resistant to beta-lactam and fluoroquinolone antibiotics. Our study's findings on the frequency of ESBL producers in swine rectal and nasal swabs provide more evidence in favor of the theory that animals might operate as reservoirs or infectious sources for the spread of these bacterial diseases [33].

The tested antibiotic was resistant to the ESBL-producing *E. coli* from swine samples in varied degrees. High resistance profiles to amoxicillin/clavulanic acid (76.9%), cefepime (92.3%), Ceftazidime (84.6%), Nalidixic acid (92.3%), and Piperacillin/Tazobactam and Cefoxitin (100.0%) were displayed by the ESBL-producing *E. coli* isolates from rectal swabs. The Meropenem-susceptible (100.0%) ESBL-producing *E. coli* isolates from nasal swabs were resistant (100.0%) to amoxicillin/clavulanic acid, cefepime, cefoxitin, colistin, and nalidixic acid. This is consistent with research conducted by [34, 35, 36]; these studies examined the antibiotic resistance pattern/multidrug resistance index of water-borne bacteria isolated from underground well water of some wards in Gusau Metropolis Zamfara State, Nigeria; additionally, they examined the multiple-antibiotic resistance and presence of CTX-M genes among Enterobacteriaceae isolates from various sources in Iwo, Osun State, Nigeria; additionally, [27], reported that bacterial isolates showed varying patterns of resistance to some commercial antibiotic disks in their antibiotic susceptibility profile of bacteria isolated from drinking water sources in Delta State Nigeria.

The widespread recovery of Multi drug resistance (MDR) ESBL *E. coli* isolates from both swine and their breeding settings on different farms in Ohaukwu local council of Ebonyi State indicates a concerning antimicrobial resistance status on pig farms, according to our study. The ESBL-producing *E. coli* isolates from swine samples in our investigation (Table 7) were multidrug-resistant (MDR) with an average score of 0.66 and a multi-antibiotic resistance index ranging from 0.33 to 0.83. The findings of [32] and [34]) are supported by this value. The isolates coded AR21, AR43, BR18, and BR34 had an antibiotic resistance index with CTX-CAZ-FEP-CT-CRO-AMC-FOX-TPZ-NA, as shown in Table 7. As seen in the Table, several isolates displayed various antibiotic resistance indices. Overall, the profile of antibiotic susceptibility of *Escherichia coli* obtained from swine rectal swab samples showed that isolates from Farms A and B were susceptible to Meropenem (100.0%) but highly resistant to Amoxicillin/Clavulanic acid (100.0%), Cefepime (100.0%), and Ceftazidime (100.0%). All of the *E. coli* isolates from Farm C were sensitive to meropenem and showed antibiotic resistance ranging from 13.8% to 100.0%. As indicated in Table 5, the Farm D isolates were 100.0% sensitive to Amikacin, Meropenem, and Ofloxacin and 100.0% resistant to amoxicillin/Clavulanic acid, cefepime, and cefoxitin. The *E. coli* isolates obtained from nasal swabs of swine in Farm B exhibited 100.0% sensitivity to Meropenem and 100.0% resistance to Amoxicillin/Clavulanic acid, Cefepime, and Ceftazidime. The isolates from Farm C were all sensitive to meropenem and ranged in antibiotic resistance from 25.0% to 100.0%. Table 6 shows that the isolates from Farm D exhibited a 100% resistance to Ceftriaxone, Ceftazidime, Colistin, and Cefepime.

Our study's results corroborate those of other studies [9, 32, 37, 38, 39, and 40] that have documented an alarmingly high rate of antibiotic resistance among bacteria, particularly *E. coli* from swine, cattle, and other animals, to last-resort drugs like colistin and carbapenems.

Previous research indicates that between 58% and 95% of ESBL-producing *E. coli* from cow faecal samples were resistant to cephalosporins [41]. The area's inadequate infection prevention and control practices combined with the misuse of antibiotics may have contributed to the emergence of this MDR index.

An international health concern is the recent surge in the development of extended-spectrum beta-lactamases by enteric bacterial isolates from various sources. A high prevalence of human infections is caused by the existence of extended-spectrum beta-lactamases (ESBLs) genes, which impart multi-drug resistance capacities up to the third and fourth generations of cephalosporins, monobactams, and carbapenems [27] and [42]. Commensal *E. coli* may be an

important source of resistance genes, as evidenced by the high frequency of extended-spectrum b-lactamases (ESBL)-producing *E. coli* in commensal isolates from animals that appear to be in good condition [43]. For the health of humans, swine, and other animals, extended-spectrum beta-lactam antibiotics—particularly the third- and fourth-generation cephalosporins—are crucial antimicrobial agents. On the other hand, Enterobacteriaceae that produce ESBLs have presented significant difficulties in clinical settings [44, 45]. Thus, this study supports earlier findings that the majority of animal-sourced bacteria isolates are developing resistance to significant antibiotics that are utilized by medical professionals. Further research is necessary to comprehend the prevalence, pathogenic arsenals, genetic relatedness/diversities, and epidemiological identities of bacterial pathogens colonizing food-producing animals, especially swine. Although MDR ESBL-producing *E. coli* from the rectal and nasal swabs of swine in our study was reported, more research is needed.

5. Conclusion

This study indicates that swine rectal and nasal swabs contained ESBL-producing *E*. *coli*. The present investigation highlights the very high frequency of MDR ESBL-producing *E. coli* isolates to amoxicillin/clavulanic acid (100%), cefepime (100%), ceftazidime (100%), and nalidixic acid (10%), among other antibiotics. The results of this study may lead one to conclude that, among other things, poor sanitation plays a significant role in the development of antibiotic resistance in the Ohaukwu Local area of Ebonyi State and that, among other things, improper waste disposal and other sanitation interventions should lower infection rates, particularly in and around animal houses.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare no conflict of interest.

Statement of ethical approval

Before any attempt to collect nasal and rectal swabs of animals used for this study was made, the protocol was approved by the ethical committee of the Ohaukwu LGA Public and Animal health following the National Health Research committee (NHREC) and the Ebonyi State university research ethics committee.

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