Characterization of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* isolates from Swine in Ohaukwu Local Government Area, Ebonyi State Nigeria

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Abstract: Antimicrobial resistant bacterial strains have become a global public health challenge affecting both humans and domestic livestock such as cattle, pork and poultry. This research work focused on the characterization of extended spectrum beta-lactamase producing *Escherichia coli* isolates from swine at Ohaukwu Local Government Area in Ebonyi State, Nigeria. A total of 400 (200 each of rectal and nasal swabs) samples were randomly collected from four swine 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. Plasmid profiling of ESBL-producing bacterial isolates was also determined. Statistical analysis was performed using SPSS 25.0 version software package. 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/culvanic acid, (76.9%); cefepime, (92.3%); ceftaxidime, (84.6%); nalidixic acid, (92.3%); and piperacillin/trazobactam and cefoxitin, (100.0%). The ESBL-producing *E. coli* isolates from nasal swabs were (100.0%) resistance to amoxicillin/culvanic acid, cefepime, cefoxitin, colistin, nalidixic acid and were (100.0%) susceptible to meropenem. The MARI index of the isolates ranged from 0.33 to 0.83 with average index of 0.66. Plasmid profiling of ESBL-producing isolates revealed presence of plasmids with the molecular weights of 850 bp to 10 kb. In conclusion, ESBL-producing *E. coli* isolates from swine was at high prevalence with presences of plasmid. Thus, threat to public health that calls for a strict measure in the choice of antibiotics used in swine productions.

Key word: Extended Spectrum Beta-Lactamase, *Escherichia coli*, Plasmid, Swine

INTRODUCTION

Extended-Spectrum Beta-Lactamase (ESBL) producers are enzymes produced by Gram-negative bacteria that bestow resistance to most beta-lactam antibiotics which include penicillins, cephalosporins, and the monobactam (aztreonam) (Silvia and Jacoby, 2014). These ESBL producers have been noticed mainly in the Enterobacteriaceae family of bacteria and the commonly encountered ones are *Escherichia coli* and *Klebsiella pneumoniae*. Beta-Lactams are a group of antibiotics acting on the cell wall of a bacterial cell. These include the penicillins, cephalosporins, carbapenems and monobactems. These bind to and inhibit the carboxy peptidases and transpeptidases. (Livermore and Paterson, 2006). These are the cell wall synthesizing enzymes, also called the penicillin-binding proteins, or PBPs, that catalyze the D-ala cross linkages of the peptidoglycan wall that surrounds the bacterium. As a result, there is weakening of the cell wall structure, leading to cell lysis. Beta-lactams, such as penicillins, carbapenems, monobactams, and cephalosporins account for 60% (by weight) of all antibiotics used worldwide, and in human medicine is one of the most widely prescribed antibiotic classes (Livermore and Woodford, 2006; Peirano, 2019). Intensive use and misuse of β-lactam antibiotics both in human and in veterinary medicine has led to the spread of extended spectrum β-lactamase (ESBL) producing resistant bacteria. The World Health Organization (WHO) has indicated that third generation

resistant Enterobacteriaceae, including ESBL-producing Enterobacteriaceae, are among the world's most serious and critical threats of the 21st century (WHO, 2017). Beta-lactams interfere with the synthesis of the bacterial cell wall, which results in the inhibition of bacterial growth, by binding to penicillin-binding-proteins (PBPs) that are enzymes involved in the synthesis of peptidoglycan. Beta-lactam antibiotics are used to treat infections caused by both Gram-positive and Gram-negative bacteria. They all share as a common structure; a four-membered lactam ring, known as the β-lactam ring. This is a cyclic amide with a hetero atomic ring structure that consists of three carbon atoms and one nitrogen atom (Smet *et al.,* 2010).

The intensive use of β-lactam antibiotics for the past 70 years has led to the evolution of β-lactam resistance bacteria. Resistance against β-lactam antibiotics in bacteria can be ensured through three different mechanisms. The first mechanism includes the mutation in genes encoding for PBPs, the creation of mosaic PBPs or obtaining alternative PBPs. The second mechanism consists of changes in the permeability of the cell wall that could be due to alterations in the expression of porins or active efflux pumps. However, the most frequent mechanism is the third one the inactivation of the antibiotic by the expression of β-lactamases (Smet *et al.,* 2010). The first β-lactamases were discovered in 1940 by Edward Penley Abraham. (Abraham and Chain, 1940). Beta-lactamases are enzymes produced by bacteria that inactivate the β-lactam ring by breaking the amide bond of the β-lactam ring and adding a water molecule to the ring-opened molecule. Narrow spectrum β-lactamases are also called penicillinases or cephalosporinases, depending on the target (Bradford, 2001; Pitout and Laupland, 2010; Blair *et al*., 2015). Moreover, some Gram-negative bacteria, especially members of the Enterobacteriaceae family, and Gram-positive bacteria, example., *Staphylococcus aureus*, can also produce an array of extended spectrum β-lactamases (ESBLs), enzymes that hydrolyze many different β-lactams and can cause resistance to oxyimino-cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefepime) and monobactams (aztreonam), but not to cephamycins (cefoxitin, cefotetan) or carbapenems (imipenem, meropenem, ertapenem, doripenem) (Pitout and Laupland, 2010; Blair *et al.,* 2015). Extended Spectrum Beta-Lactamases are inhibited by ESBL inhibitors, such as clavulanate, sulbactam and tazobactam (older β-lactamase inhibitors) and avibactam, relebactam and vaborbactam (latest Food and Drug Administration (FDA)-approved inhibitors), which are therefore frequently included with β-lactam antibiotics in the formulation of therapeutic drugs (Bush and Bradford, 2020).

As β-lactamases share some sequence homology with PBPs, it is considered that they have evolved from them (Medeiros, 1997; Bradford, 2001). Beta-lactamase encoding genes (*bla*) can be located in chromosomes or plasmids. The first plasmid-mediated β-lactamase (TEM-1) in Gram-negatives was described in 1965, in an isolate from a patient named Temoniera in Greece, therefore named TEM (Datta and Kontomichalu, 1965)**.** Since then, hundreds of different β-lactamases have been discovered, and the first ESBL (SHV-2) was discovered in Germany, isolated from a *Klebsiella ozonae* strain in 1985. The CTX-M enzyme was discovered in 1985 from *Klebsiella pneumoniae*, isolated from patients in an intensive care unit in France (Sirot *et al.,* 1987). Nowadays, it is the most widely spread β-lactamase in food-producing animals (Irrgang *et al.,* 2018). According to the reports of the European Food Safety Authority (EFSA) the prevalence of presumptive ESBL producing *E. coli* in fattening pigs and pork meat varies a lot within the EU countries (EFSA, 2019). It is worth to note that there are several steps within the pork production chain where pigs are exposed to ESBL producing bacteria and can become carriers

of them, such as trading places, where new animals are mixed with older animals within the same herd, or slaughterhouse waiting areas (Schmithausen *et al.,* 2018). In addition, cross-contamination in slaughterhouses, especially at evisceration, poses a risk of carcass contamination with ESBL producing Enterobacteriaceae (Warriner *et al.,* 2002; Wu *et al.,* 2009). This research work focused on the characterization of extended spectrum beta-lactamase producing *Escherichia coli* from swine at Ohaukwu Local Government Area in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in Ohaukwu Local Government Area of Ebonyi State, Nigeria. 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.

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, Nigeria. 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 within two hours of collection for routine microbiological analysis. Then samples were cultured on nutrient broth for isolation of *Escherichia coli.*

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 24 h. Suspected *Escherichia coli* from positive cultures were identified by their characteristic appearance (colour, 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 (Cheesbrough, 2006).

Antibiotic susceptibility testing of the bacterial isolates: The antibiotic susceptibility patterns of the identified isolates were determined by disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI, 2008) 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), ceftaxidime (30 µg), colistin (10 µg), meropenem (10 µg), nalidixic acid (30 µg), ofloxacin (5 µg) and piperacillin/trazobactam (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 10 minutes and were incubated at 37^0C for 18-24 h. The diameters of zones of inhibition were measured in millimeter (mm) with rule, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019).

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-generationcephalosporins (such as cefotaxime and ceftriaxone) using the double disk synergy test (DDST) technique (Ugwu *et al*., 2019). Standardized inoculums of the isolate (adjusted to 0.5 McFarland turbidity standards) was 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 37ºC for 18 - 24 h; 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 (Ugwu *et al*., 2019).

Plasmid deoxyribonucleic acid (DNA) extraction of the bacterial isolates: *Escherichia coli* isolates grown for 24 h in 5 ml of LB broth (Merck, Germany) were harvested by centrifuging 1.5 ml of each culture in certified microcentrifuge tubes (China, MCT-150, ISO9001) for 4 minutes at 4000 rpm (revolutions per minute) and the supernatant was discarded. The plasmid DNA was extracted using Zyppy Plasmid Miniprep Kit (Zymo Research, Epigenetics, USA) according to the manufacturer's instruction. The resulting plasmid preparations were stored at -20^0C for further use (Dutta *et al*., 2020).

Agarose gel electrophoresis: Plasmid DNA was determined in a 1% (w/v) agarose gel by dissolving 1.0 g of agarose (Bio-Rad) in 100 ml of1 X Trisacetate-ethylene diamine tetraacetate (TAE; pH 8.0) buffer. The mixture was heated for 3 min in a microwave oven. After cooling, ethidium bromide (EtBr; 1 ml/ml) was added to the molten gel, which was then poured in agel casting tray and then allowed to solidify. After solidification, combs were removed and the gel was carefully placed in the electrophoresis tank containing1X TAE buffer (40 mMTris, 20 mM acetic acid, and100 mM EDTA pH 8.0). The plasmid DNA detection were prepared by mixing 5µl of plasmid DNA extract with 2µl of 6 X DNA loading dye (Fermentas). For each run, 5 µl of Lambda DNA/HindIII Marker 3 (2.5 kb; Thermo Fisher Scientific) was added to one of the wells to estimate the band sizes and 5µl of negative control, comprised of Sigma water (Nuclease free water) was added to another well. Then 5 µl of the extracted plasmid DNA of each isolate were carefully loaded into the remaining wells. Agarose gel electrophoresis was performed at 80 V; 400 mA (mini-Ampere) for 60 minutes. Gels were visualized and photographed using agel documentation system (Gel Doc 2000; Bio-Rad) (Normand *et al.,* 2000; Brody and Kern, 2004).

Data analysis of the bacterial isolates: Data generated from this research were analyzed using statistical package for social sciences (SPSS) version 25.0 software. One way ANOVA and chi-square test were used to determine the prevalence base on the swine type. Results were taken as significant where *p* value is less than 0.05 ($p < 0.05$).

RESULTS

Out of 400 samples collected, 164(41.0%); 140(35.0%) and 96(24%) were obtained from sow/piglets; weaners and finishers respectively. A total of 157(39.3%) were positive for *Escherichia coli* and a total of 85(54.1%) and 72(45.9%) of the isolates were identified from rectal and nasal swabs respectively. There was significant difference in the prevalence of *E. coli* at ($p < 0.05$) in relation to samples type (Table 1). Distributions of *E. coli* were recorded according to type of the swine; sow/piglets and weaners had the highest number of the isolates while the finishers had the least occurrence. Statistical analysis showed a significant change in the *E. coli* prevalence at ($p<0.05$) (Table 2). The findings showed that 19 (12.1%) isolates were beta-lactamase producers (Table 3) while 13(15.3%) were found to be from rectal swabs and 6(8.3%) from nasal swabs. There was significant difference in the prevalence of ESBL-producing *Escherichia coli* at ($p < 0.05$) (Table 4). Plasmids were identified in 14 (73.7%) among the isolates with the molecular size ranging from 1500 bp to 10 kb (Figure 1). *Escherichia coli* isolates showed different range of resistant and susceptibility to different classes of antibiotics tested. Beta-lactamase producing *E. coli* were highly resistant to beta-lactam drugs with increasing percentage range; ceftriaxone (33.8 to 52.4), ceftazidime (37.2 to 63.5) and cefotaxime (41.3 to 77.4). Notably, all the ESBL producing *E. coli* strain was susceptible to meropenem, although, some of the strains of *E. coli* were meropenem resistant which ranged from 5.2 to 23.4 percent (Table 5).

Table 1: Frequency of swine samples collected with respect to swine type in Ohaukwu Local Government Area

Swine Type	Farm				Total
	A	B	C	D	
Sow/Piglets	64	46	12	42	164
Weaners	10	54	36	40	140
Finishers	26	0	52	18	96
Total	100	100	100	100	400

Table 2: Frequency of isolation of *E. coli* from rectal and nasal swab samples of swine in Ohaukwu Local Government area of Ebonyi State, Nigeria

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

Table 3: Distribution of ESBL-producing *E. coli* from Rectal and Nasal swab of swine in Ohaukwu Local Government Area of Ebonyi State, Nigeria

Sample type	No. of Samples	No. +ve for E. coli (%)	ESBL-positive (%)
Rectal swab	200	85(42.5)	13(15.3)
Nasal swab	200	72(36.0)	6(8.3)
Total	400	157 (39.3)	19 (12.1)

Table 4: Plasmid profile of ESBL genes in *E. coli* isolates from rectal and nasal samples of swine in Ohaukwu Local Government Area of Ebonyi State, Nigeria

Sample type	Sample source - Farm	Bacteria coding	Plasmid profile	Plasmid size (bp / kb)
	A	AR15	+	1500 bp, 10 kb
	A	AR21	+	2000 bp
	A	AR27	+	1500 bp
	A	AR43	+	1500 bp
	B	BR18	+	850 bp, 10 kb
	B	BR34	+	1500 bp
Rectal swab	D	DR31	+	850 bp, 1500 bp
	D	DR40	+	850 bp, 1500 bp
	D	DR45	+	1500 bp
	Total	(n=9)		
Nasal swab	B	BN10	+	1500 bp, 10 kb
	B	BN14	+	1500 bp, 10 kb
	B	BN26	+	1500 bp, 10 kb
	C	CN30	+	850 bp, 1500 bp
	D	DN45	+	1500 bp
	Total	(n=5)		
Overall total		n = 14		

Table 5: Antibiotic susceptibility profile of ESBL producing *E. coli* from swine samples of farms in Ohaukwu Local Government Area of Ebonyi State, Nigeria

Sample type	Rectal swab		Nasal swab	
	E. coli (n=13)		*E. coli* (n=6)	
Antibiotics (µg)	R (%)	S (%)	R (%)	S (%)
Amikacin (15)	5(38.5)	8(61.5)	2(33.3)	4(66.7)
Amoxicillin/Culvanic acid (30)	10(76.9)	3(23.1)	6(100)	0(0.0)
Cefepime (10)	12(92.3)	1(7.7)	6(100)	0(0.0)
Cefotaxime	4(30.8)	9(69.2)	1(16.7)	5(83.3)
Cefoxitin (30)	13(100)	0(0.0)	6(100)	0(0.0)
Ceftriaxone (30)	3(23.1)	10(76.9)	1(16.7)	5(83.3)
Ceftaxidime	11(84.6)	2(15.4)	4(66.7)	2(33.3)
Colistin (10)	13(100)	0(0.0)	6(100)	0(0.0)
Meropenem (10)	0(0.0)	13(100)	0(0.0)	6(100)
Nalidixic Acid (30)	12(92.3)	1(7.7)	6(100)	0(0.0)
Ofloxacin (5)	0(0.0)	13(100)	0(0.0)	6(100)
Piperacillin/Trazobactam (85)	13(100)	0(0.0)	3(50)	3(50)

Key: R-Resistance, S- Susceptible, n=Number of isolates

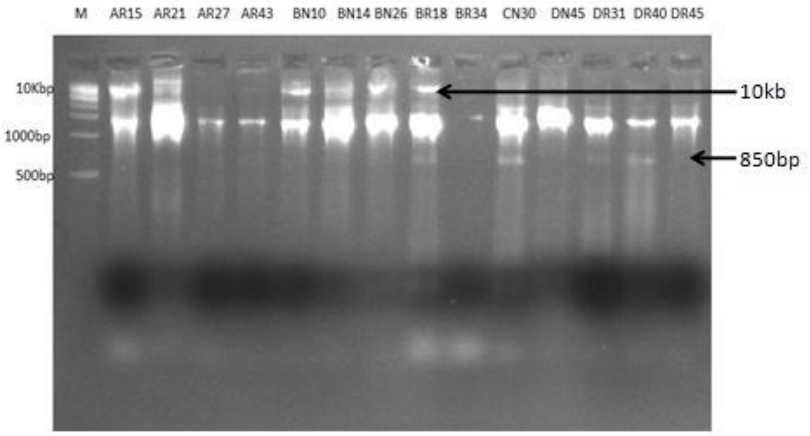

Plate 1: Gel electrophoretic separation profile of plasmid DNAs isolated from ESBL-producing *E. coli* isolates

Key: Lane M = 10kb HindIII Marker 3, Lane: AR15, AR21, AR27, AR43, BN10, BN14, BN26, BR18, BR34, CN30, DN45, DR31, DR40 and DR45 = *E. coli* plasmid amplicons

DISCUSSION

Out of 400 swine samples collected, sow/piglets have 164(41.0%), the weaners 140(35.0%) while the finishers were 96 representing 24%. Therefore, 64(39.0%); 46(28.0%); 12(7.3%) and 42(25.6%) of the samples were from sow/piglets swab collected from farm (A, B, C and D) respectively and the weaners swab were 10(7.1%); 54(38.6%); 36(25.7%) and 40(28.6%) from farm (A, B, C and D) respectively while the finishers swab were 26(27.1%); 0(0.0%); 52(54.2%) and 18(18.8%) from farm (A, B, C and D) respectively. This research has shown the prevalence of *E. coli* from rectal and nasal swabs of swine as 157 (39.3%) out of the 400 samples studied. This report and the reports of Liu *et al.*, (2018) and Ajayi *et al.*, (2023) are in agreement. In several farms where swine were isolated, the prevalence of *Escherichia coli* was found in rectal swabs 85 (54.1%) and nasal swabs 72 (45.9%).

The frequency of *Escherichia coli* in different farms isolated from swine was highly prevalence in sow/piglets 63(40.1%) followed by weaners 57(36.3%) while the finishers 37(23.6%) had the least occurrence. Therefore, 17(26.9%); 17(26.9%); 8(12.6%) and 21(33.3%) of the *E. coli* isolates were from sow/piglets swab collected from farm (A, B, C and D) respectively. Also, the weaners swab harboured 6(10.5%); 11(19.3%); 20(35.1%) and 20(35.1%) of the *E. coli* isolates from farm (A, B, C and D) respectively while the finishers swab harboured 6(16.2%); 0(0.0%); 21(56.6%) and 10(27.0%) of the *E. coli* isolates from farm (A, B, C and D) respectively.

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 Local Government Area of Ebonyi State, Nigeria. According to table 3 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 producing *Escherichia coli* in the samples. This finding is consistent with that of Egwu *et al.,* (2024), who found that *Escherichia coli* isolated from animals that produce food was resistant to beta-lactam and fluoroquinolone antibiotics. The 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 (Carattoli, 2008). This isolate have been documented to transmit plasmids containing the gene encoding ESBL from the natural environment to humans or livestock (Dohmen *et al.*, 2017). This report provides insights into the distribution and occurrence of ESBL-producing bacteria in different regions and animal populations, helping to fill gaps in our understanding of this important issue.

Antibiotic susceptibility profile of ESBL-Producing *E. coli* isolates indicated a high level of resistance to most antibiotics studies. The unique feature of *E. coli* isolates is the resistance of variety of antibiotics, The ESBL-producing *E. coli* isolates from rectal swab showed high resistant profile to amoxicillin/culvanic acid, (76.9%); cefepime, (92.3%); cefoxitin, (100.0%); ceftaxidime, (84.6%); nalidixic acid, (92.3%); and piperacillin/trazobactam, (100.0%). The ESBL-producing *E. coli* isolates from nasal swabs had (100.0%) resistant profile to amoxicillin/culvanic acid, cefepime, cefoxitin, colistin, nalidixic acid,

and piperacillin/trazobactam, (50.0%) ceftaxidime, (66.7%). Extended Spectrum Beta-Lactamase producing *E. coli* isolates from swine had resistant range of 53.0%-100% to ampicillin, ticarcillin, cefalexin, cefepime, trimethoprim, sulfametaxazole-trimetoprim, gentamicin,tetracycline, enrofloxacin, cefazolin, cefotaxime, cefepime, andamoxicillin-clavulanic acid (Galin *et al*., 2021) and similar report in Uganda reported that *E. coli* isolates from the pigs slaughter houses showed higher prevalence of multidrug resistant *E. coli* isolates as follows; amoxicillin (30.4%), erythromycin (34.8%), streptomycin (40.7%), ciprofloxacin(100%), oxytetracycline (31%) and sulphamethoxazole-trimethoprim (42.9%). This is consistent with research conducted by Magiorakos *et al.,* 2012; Strom *et al.,* 2017; This may be as a result of irrational and inappropriate use of antibiotics by swine farmers which contributes greatly to bacterial resistance to antimicrobial agents (Smet *et al.,* 2010).

The established fact is that stem from an increased worldwide distribution of ESBL producers *E. coli* in food producing animals conferring resistance to β-lactam antimicrobials (Islam *et al*., 2023). These antimicrobial agents used in the treatment of food animals in veterinary medicine range from penicillins, first to fourth generation cephalosporins and β-lactamase inhibitors (Li *et al*., 2007). The ESBL-producing *Escherichia coli* are also resistant to most antibiotics such as aminoglycosides, tetracyclines, chloramphenicol, trimethoprim, sulphonamides and quinolones (Hunter *et al.,* 2010). The cause of disease in swine by bacterial agents is a problem that is often faced by managers of swine farms and this is responsible for the development of resistant against *E. coli* to routinely used antibiotics. This has led to the use of antibiotics for the prevention and treatment of the diseases (Van *et al.,* 2019). The ESBL-producing *E. coli* from swine samples in this study showed varying range of resistance to the antibiotic tested on them and the irrational use of antibiotics by swine farmers contributes greatly to bacterial resistance to antimicrobial agents (Islam *et al*., 2023). The harmful impact caused by bacterial resistance to antibiotics is that the treatment time for bacterial diseases becomes longer or the treatment fails. Less effective treatment impacts the length of treatment and the use of drugs that are more expensive and, of course, the costs incurred (Soraas *et al.*, 2014).

WHO, reported that most countries have resistance between 10% and 25%, while more than 25% are found in Bulgaria, Cyprus, Italy, and Slovakia (WHO, 2017). Among Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) countries, the reported resistance exceeds 50% (Montenegro, Russia, Northern Macedonia, and Turkey), while in Serbia it varies between 25% and 50% (WHO, 2019). In Nigeria, antimicrobial resistance in *E. coli* has been reported to range from 23.8% to 82.6% (Ugbo *et al*., 2020). The *E. coli* isolated from swine were found to show resistances to amoxicillin (75%), tetracycline and chloramphenicol (56.25%), trimethoprim/sulfamethoxazole (43.75%), doxycycline hydrochloride (37.5%), nalidixic acid (25%) and ampicillin (68.75%) (Dimitrova *et al*., 2016). In a similar study, findings showed that the high level of resistance was dominated by erythromycin at 85.4% and prevalence of multi drug resistant *E. coli* isolates in pig farms was 57.3% (Arruda, 2004). Another researcher who obtained samples from food animals such as chicken, pigs, cattle, goats and sheep and reported highest resistance against erythromycin at 96.0%, tetracycline at 61% and the least resistance was in ciprofloxacin at 6.5% (Byarugaba *et al*., 2011). A retrospective study among 63 archived *E. coli* samples from poultry between 2012 and 2018 showed that multidrug resistance among 43 recovered *E. coli* samples was at 88.4%. (Kakooza *et al*., 2021). Multidrug resistant bacteria have been reported in other livestock other than swine especially among pigs and dairy cattle

(Byaruhanga *et al*., 2022). Antimicrobial are still in use as growth promoters in some countries on farm animals such pigs, cattle, poultry birds and has not yet prohibited the use of antibiotics as growth promoters, and this has contributed greatly in the dissemination of antimicrobial resistance in livestock (Yang *et al*., 2019; Xiao, 2020).

The majority of the ESBL-producing *E. coli* isolates were found in rectal swab in this study. The high prevalence observed in rectal may be due to frequent administration of antimicrobial drugs to swine animals which in turn increases the risk of higher antimicrobial resistant *E. coli* strains in the normal intestinal flora. This is in agreement with a study conducted by Carattoli, (2008) which established that swine were possible reservoirs for resistant fecal flora, particularly *E. coli*. This high colonization rate could be attributed to cross contamination of swine food products and antibiotics products particularly during feeding and treatments which is a potential risk factor that could exacerbate the transmission rate of ESBL-producing *E. coli* resistant genes (Diop *et al.,* 2012; Cook *et al.,* (2017). In this study the factors responsible for the high levels of ESBL-producing *E. coli* isolates was not fully known as the study did not focus on the potential risk factors. A researcher previously reported moderate rates of the resistance of *E. coli* isolated from swine to norfloxacin (43.0%), ciprofloxacin (47.6%), ofloxacin (47.0%), and levofloxacin (38.8%) (Cheng *et al*., 2020). A major mechanism of cephalosporin resistance is the production of beta-lactamases, which hydrolyzes the beta-lactamring and inactivates beta-lactam chemotherapeutics. The genetic determinants of resistance demonstrated in *E. coli* isolates include extended-spectrum beta-lactamases (ESBLs) encoded by various plasmid genes (*bla*SHV, *bla*CMY-2, *bla*TEM, *bla*CTX-M, *bla*OXA and others), as well as a number of gene area relationships (GARs) for quinolone resistance (qnr), trimethoprim (dhf), aminoglycosides (aac) (Buranasinsup *et al*., 2018; Effendi *et al*., 2022).

The most disturbing pattern observed in this study was the multiple antibiotic resistance index (MARI) of *E. coli* isolated from rectal swab and nasal swab showing multidrug resistant with MARI value of 0.33 to 0.83 with average index of 0.66. The isolate were non-susceptibility to at least one antibiotic in at least three classes for which *E. coli* susceptibility is generally expected. The swine swabs at greatest risk of acquiring MDR- *E. coli* as observed in this study is rectal swabs of swine. 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 such as CAZ-FEP-CT-CRO-AMC-FOX-NA; CTX-CAZ-FEP-TPZ and CTX-CAZ-FEP-AMC-FOX-TPZ-NA. The MDR *E. coli* isolates from swine has been reported to show resistance to the following antibiotics; aminoglycosides, streptomycin, gentamicin, penicillins, fluoroquinolones, macrolides (Magiorakos *et al.,* 2012; Dimitrova *et al*., 2016) which agreed with this study. The development of high antibiotic resistance profile in *E. coli* isolated from swine could be attributed to frequent misuse of β-lactum antibiotics in treatment and management of swine diseases and also the uncontrolled use of antibiotics as growth promoters in swine farms (Johnson *et al.,* 2006). Studies has stated that MDR-*E coli* is a serious threat to animal and human health and causes a disease that often occurs in swine from birth to weaning, characterized by white to yellow diarrhea, disease such as colibacillosis (Magiorakos *et al.,* 2012). Antibiotic-resistant *E. coli* can be spread from animals to humans through the food chain, direct contact, or the environment (Schwaiger *et al*., 2021). One factor that contributes to promoting the antimicrobial resistance (AMR) phenomenon is the fact that *E. coli* presents a high capacity to acquire and pass antimicrobial resistance genes via horizontal gene transfer (Poirel *et al*., 2018). The *E. coli* isolates revealed an alarming scenario

with high resistance to a different antimicrobial class, as is the case of penicillins, aminoglycosides, tetracyclines, sulphonamides, fluoroquinolones, and phenicols. In 2019, *E. coli* was considered one of the major pathogens responsible for the deaths associated with AMR (Murray *et al*., 2022). There is an increasing trend in the detection of AMR among *E. coli* from swine with enteric colibacillosis (Luppi, 2017). As such there is a substantial need for evaluation of wide spectrum and new therapies in different classes to counteract this imminent crisis of resistance among ESBL-producing *E. coli.*

CONCLUSION

This study reports the high prevalence of *E. coli* from rectal and nasal swabs of swine in Ohaukwu Local Government Area of Ebonyi State, Nigeria. The ESBL-producing *E. coli* isolates had high prevalence in sow/piglet 10(52.6%) followed by weaners 7(36.8%) while finishers 2(10.5%) had least occurrence and ESBL-producing *E. coli* from swine had high prevalence of the various ESBL-encoding genes studied. The presence of plasmid associations were reported, and thus a big threat to public health.

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