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Antimicrobial Resistance among Enterobacteriaceae Found in Chicken and Cow Droppings and Their Public Health Importance

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Abstract

Introduction: The recent surge in the number of antimicrobial resistant cases from hospitals and communities has created a need to study the points and sources of exposure to certain bacteria and determine their susceptibility to commonly used antibiotics. This study aimed at identifying and screening for drug-resistant Enterobacteriaceae isolated from chicken droppings and cow dungs in Onitsha, Anambra state, in the South-Eastern part of Nigeria. **Methods**: This is a cross-sectional descriptive study which included 50 chickens and 50 cow dung samples collected from five poultry houses and cow ranches respectively using sterile swab sticks. The samples were transported to the laboratory and processed following standard microbiological protocols. Isolates in the samples were recovered using MacConkey Agar, Eosin Methylene Blue Agar and Salmonella-Shigella Agar following standard microbiological procedures and then identified/characterized biochemically using commercial API 20E identification kits following the standard manufacturer's protocol. Isolates were subjected to antibiotic susceptibility testing on Muller Hinton Agar using Kirby Bauer double-disc diffusion technique. The multiple antibiotics resistance index was determined as well. Isolates with reduced susceptibility to Ceftazidime were screened for extended spectrum beta-lactamase, AmpC- and metallo-beta-lactamase-production using Rosco Diagnostic kit. **Results**: Sixty-two (100%) Gram-negative bacteria were isolated from a total of 100 samples collected from both sites, out of which 43 (69.4%) are Enterobacteriaceae. A total of 30/43 (69.8%) Enterobacteriaceae including *K. pneumoniae*, *S. enteritica*, *S. odorifera*, *E. coli*, *K. intermediate*, *P. stuartii*, *E. aero-*

genes, *P. penneri*, *P. mirabilis and C. braakii* were recovered from chicken droppings, whereas 13/43 (30.2%) Enterobacteriaceae including *K. pneumoniae*, *S. enteritica*, *S. odorifera*, *E. coli*, *K. intermediate*, *P. stuartii*, *E. aerogenes*, *P. penneri*, *P. mirabilis and C. braakii* were recovered from cow dungs. Two (12.5%) different isolates demonstrated metallo-beta-lactamase and cephalosporinase (AmpC) production. The isolates were susceptible to six antibiotics tested except Augmentin and Nitrofurantoin where the resistance is 100% and 85% respectively while Ceftriaxone and Ofloxacin had the best antibacterial activity against the isolates from both sites. **Conclusion**: The bacteria of public health importance isolated from these sites and their antibiogram profile have shown the need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health as well as contribute to the fight against antibiotic resistance.

Keywords

Antibiogram, Antibiotic Resistance, Enterobacteriaceae, One Health, Nigeria

1. Introduction

The Enterobacteriaceae, a family of aerobic and Gram-negative rods that naturally inhabit the intestinal tract of humans and animals, have been implicated in many human diseases. This family of bacteria has recently been experiencing a rise in incidence of resistance to antibiotics, as reported in many countries of the world, thus posing a bigger threat to healthcare delivery [1] [2]. They are particularly of clinical importance in the cause of nosocomial and community acquired bacterial infections. With the continued economic activities in poultry practice, cattle ranching and increased exposure of both crop fields and humans to antibiotic-resistant bacteria present in chicken and cow excreta, human contacts with enterobacterial infections are inevitable and have constituted a threat to public health [3].

More so, previous studies have attributed the irrational use of antibiotics in the practice of animal husbandry as the reason for the emergence and spread of resistant bacteria [3] [4]. Resistance of the Enterobacteriaceae to commonly used antibiotics in the last decade has been of an alarming proportion, causing increased public health concerns [5] [6]. The mechanisms of resistance to such antibiotics are usually through efflux pumps, enzyme modification of the antibiotic, selective pressure and antibiotic inactivation [4] [5] [6] [7]. Commonly used antibiotics are becoming less useful owing to resistance and most of the antibiotics considered as last resort are also becoming ineffective for the same reason [8].

Furthermore, carbapenems are β-lactam group of drugs that are currently used as antibiotics of last resort for treating infection because of the problem of multidrug-resistance especially among Gram-negative rods [2] [9] [10]. This re-

sistance has largely been attributed to the production or acquisition of Carbapenemases among enterobacteriaceae family [11]. Originally, organisms belonging to the *enterobacteriaceae* family were susceptible to carbapenems, but this is no longer true due to the emergence of Carbapenem resistant *enterobacteriaceae* in the last couple of years and so posing a serious health concern [10]. The Center for Diseases Control and Prevention (CDC) in 2013 reported that Carbapenem-resistant enterobacteriaceae (CRE), which emerged within the past two decades, among other multidrug-resistant *organisms*, have remained the major cause of untreatable and hard-to-treat infections among hospitalized patients, and are considered an urgent threat to human health [2] [10]. Detecting CRE early in human and animal hosts is highly recommended in controlling infections by them as well as their spread [2].

Consequently, contamination of food and food-producing animals with MDR bacteria harboring MBLs and AmpC enzymes could be a source of antibiotic resistance [12]. Over the last few decades, several extended-spectrum *β*-lactamases (ESBL) and AmpC-producing Enterobacteriaceae (EPE) have emerged in both human and animal health management globally, with the animals being touted as the transmission link of ESBLs/AmpCs for humans [10] [11] [13]. In addition to this, Ejikeugwu *et al.*, reported AmpC producing Enterobacteriaceae, as well as MDR and production of MBL amongst *Klebsiella* spp isolated from cow anal swabs in studies carried out in Nigeria [12] [14]. Although some studies have been carried out in Nigeria at poultry and animal houses, yet paucity of data is available on antimicrobial resistance resulting from poultry droppings and cow dungs especially in South-Eastern Nigeria; thus, creating a research gap for this study. In this study, we proposed the hypothesis that antibiotic resistance in Enterobacteriaceae recovered from both poultry droppings and cow dungs could be attributed to intrinsic genetic factors possessed by these organisms that enhance the production of MBL and AmpC enzymes. Hence, this study was aimed at identifying and screening for drug-resistant Enterobacteriaceae isolated from chicken droppings and cow dungs in Onitsha, Anambra state, in the South-Eastern part of Nigeria.

2. Methods

2.1. Study Setting

This is a cross-sectional descriptive study conducted from August, 2020 to April, 2021 at Nkwelle suburb, Onitsha North Local Government Area, Onitsha. Onitsha is a metropolitan city located near the River Niger, in Anambra state, South-Eastern of Nigeria. It lies within 6˚10'N 6˚47'E coordinates in a 36.12 km^2 landmass, and has a population of 561,066 [15].

The study included five different poultry and cow ranches with a capacity of 500 birds and 200 cows respectively. Simple random sampling was used to collect swabs of 50 chicken droppings and 50 cows dungs respectively. Information obtained from the farm attendants showed that, although, proper hygiene is

maintained in the poultry and ranch. The livestock are usually given growth enhancement feeds and water containing antibiotics alongside routine veterinary checks, including medical treatments.

Collection and transportation of fecal Samples

Four heaped dessert-spoonful of fresh, warm dung pat on the ground samples were collected from adult cattle from 5 different areas where adult cows congregate. They were mixed and then a sterile swab stick was used to rob through the mixture.

Fifty (50) chicken and cow dung swab samples each, were collected from the poultry house and cow ranch in Nkwelle, Onitsha using sterile swab sticks and early in the morning before the attendants came for their routine cleaning and tending of the chicken and cows. Samples were collected by robbing a wet sterile swab stick on chicken and cow excreta dropped at different locations of the same poultry and ranch, for five different places. Each day, the collected samples were transported aseptically and processed within 2 hours of collection. The samples were processed at laboratory in Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Agulu of Nnamdi Azikiwe University, Awka.

Culture and purification of fecal Samples

The chicken dropping and cow dung swab samples were each cultured in 5 ml double strength of nutrient broth (CM0003, Oxoid, UK) and incubated overnight at 30˚C. A loopful of the specimen was transferred aseptically onto MacConkey agar (MAC) plates, Eosin Methylene Blue (EMB) agar, Cetrimide selective agar plates, and Salmonella-Shigella agar for the selective isolation of *Klebsiella* species, *Escherichia coli* and *P. aeruginosa* and *Salmonella-Shigella* species respectively and incubated at 30˚C for 18 - 24 hours for phenotypic characterization. Phenotypically, *Escherichia coli* produces colonies with metallic green sheen on EMB agar and lactose-fermenting colonies on MAC; *Klebsiella* species produce small, circular, elevated and mucoid colony on MAC and non-metallic sheen mucoid colonies on EMB agar while *P. aeruginosa* isolates produce greenish pigmentation on Cetrimide selective agar [16].

Identification and confirmation of bacterial isolates using BioMerieux API 20E Kit

After Gram staining and microscopic examination, the various isolates obtained were characterized biochemically using BioMerieux API-20E kit to further identify the organisms to species level. The pure cultures were further characterized using Analytical Profile Index (API) 20E test strip comprising of 20 micro-tubes seeded with dehydrated substrates for enzymes that are produced by Enterobacteriaceae family. To allow for some moisture during incubation, distilled water (5 ml) was spread onto the honey comb wells of the tray to ensure a humid environment in the incubation box before the strips were placed in the tray. Discrete colonies collected from 20 hours overnight culture plates were inoculated into 5 ml API 20E suspension medium, and emulsified to ensure a homogenous bacterial inoculum. Following the manufacturer's specifications, the

preparations were carefully distributed into the tubes of the strips, ensuring that no air bubbles were left. The tube and cupule for GEL, VP and CIT tests were filled with the suspension while the inoculum was filled up to the tube in the rest of the test segments. To create an anaerobic environment for ADH, LDC, ODC, H2S and URE tests, the tubes were overlaid with mineral oil and the set up incubated for 24 hours at 37˚C. One drop each of TDA and James reagents was added in TDA and IND tubes respectively, one drop each of VP1 and VP2 was added in VP tube after the incubation period. The results were read and recorded after 10 minutes.

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility testing was carried out on all the identified bacterial isolates using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton (MH) agar plates (Oxoid, UK) [2]. The breakpoint chosen was in accordance with the Clinical and Laboratory Standard Institute (CLSI) guideline [13]. The single disks used were: Ofloxacin 5 µg (OFL), Ceftazidime 30 µg (CAZ), Cefuroxime 30 µg (CRX), Gentamicin 10 µg (GEN), Ceftriaxone 30 µg (CTR), Augmentin 30 µg (AUG), Cefixime 5 µg (CXM), Nitrofurantoin 300 µg (NIT).

Screening for Extended spectrum-*β*-lactamase production using ROSCO kits

For the phenotypic detection of the various *β*-lactamases present in the strains, the double tablet synergy testing using subjective observations of synergy and the combination tablet method was used [13]. Attention was given to the differences in the zones of inhibition. The susceptibility tests were performed following the method M2A6 disc diffusion method on Mueller Hinton agar plate as recommended by the National Committee for Clinical Laboratory Standards [17]. A standardized (0.5 MacFrland) inoculum was swabbed onto the Mueller Hinton agar plate using sterile swabs and the discs were aseptically placed on the inoculated plates and pressed firmly onto the agar plate for complete contact while ensuring sufficient space between individual disc to allow for proper measurement of inhibition zones. The test isolates were tested against the following antibiotic discs; Meropenem (MRP10 µg), Meropenem + Cloxacillin (MRPCX), Meropenem + Clavulanate (MRPC), Meropenem + Phenylboronic Acid (MRPBO), Meropenem + Cloxacillin (MRPCX), Meropenem + DPA (MRPDP), Temocillin (30 µg), in an inverted format. The Plates were left on the work table for 30 minutes to allow for pre-diffusion of antibiotics into the agar. Afterwards, they were incubated at 37˚C for 18 - 24 hours. The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition was then interpreted following the guide stated by the manufacturer.

Screening for Amp-C *β*-lactamase production

To test for Amp-C *β*-lactamase production, the test organisms were screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30

μg) using Kirby Bauer disk diffusion. Following CLSI standard, isolates with an IZD of ≤ 18 mm were suspected to produce AmpC enzyme.

Screening for Metallo-*β*-lactamase (MBL) production

This was done by phenotypically screened for the production of MBL in the test isolates. Their susceptibility to imipenem (IPM), meropenem (MEM), and ertapenem (ETP) was also done according to the CLSI criteria. Test isolates with an inhibition zone diameter (IZD) of ≤23 mm were suspected to harbor MBL enzyme.

Determination of Multiple Antibiotic Resistance Index (MARI)

The MARI was calculated using the formula:

$$\text{MARI} = \frac{\text{Number of antibiotics to which the isolates were resistant}}{\text{Total number of antibiotics to which the isolates were subjected}}$$

The incidence of multidrug resistant isolates was calculated from the formula [15]

$$\text{Incidence of multidrug resistant isolates} = \frac{\text{Number of isolates with MARI} \geq 0.3}{\text{Total number of Isolates}} \times 100$$

2.2. Data Analysis

All the data collected was summarized and tabulated using Microsoft excel software 2016. The results were calculated using percentages and presented in tables, while the Multiple Antibiotic Resistance index (MAR index) was calculated for each isolate and tabulated in the result section.

3. Results

A total of 43 (69.4%) Enterobacteriaceae recovered from this study out of 62 (100%) Gram-negative bacteria (GNB) isolates obtained from this study. Table 1 shows the frequency of Gram-negative bacteria recovered from the samples collected from 40 (80%) chicken droppings and 22 (44%) cow dungs. *K. pneumoniae* and *E. coli* had the highest frequency in chicken droppings (15%), while *K. pneumoniae* and *E. cloacae* had the highest frequency in cow dung (18.1%). The Enterobacteriaceae recovered all showed to be lactose fermenters with pink colonies on MAC, whereas non-lactose fermenters showed pale colonies on MAC and were all negative for oxidase test. Characterization of the isolates biochemically using the API®20E kit identified the isolates accordingly (Table 1).

Table 2 and Table 3 show the antibiogram profiles of the isolates to the tested antibiotics together with their respective MARI. All the isolates from chicken dropping, except *Citrobacter braaki*, were resistant to Augmentin while the isolates *Serratia odorifera* and *Enterobacter cloacae* were resistant to all the antibiotics tested (Table 2). Ceftriaxone and Ofloxacin had the best antibacterial activity against the isolates from both sites. All the isolates from cow dungs were resistant to Augmentin while *Shewanella putrefaciens* was resistant to all the antibiotics tested (Table 2). Table 2 and Table 3 show the multidrug resistant

Table 1. Frequency of isolates identified from this study using API-20E kit.

Isolates' Source	Assigned Isolate API Number	Organisms	*Frequency (%)
Chicken Droppings (N = 40)	7214773	*Klebsiella Pneumoniae*	15.0
	5506572	*Salmonella enterica* spp *arizona*	2.5
	402000	*Shewanella putrefaciens*	7.5
	3304573	*Enterobacter cloacae*	7.5
	1504573	*Citrobacter braaki*	2.5
	5105532	*Escherichia coli*	15.0
	536000	*Proteus mirabilis*	5.0
	5737773	*Serratia odorifera*	10.0
	206020	*Burkholderia cepacia*	7.5
	1104572	*Kluyvera intermedia*	7.5
	224300	*Providencia stuartii*	2.5
	604040	*Acinetobacter baumannii*	2.5
	1205773	*Pantoea spp*	7.5
	1305773	*Enterobacter aerogenes*	5.0
	436020	*Proteus penneri*	2.5
Cow dungs (N = 22)	5214773	*Klebsiella pneumoniae*	18.2
	2302000	*Pseudomonas aeruginosa*	4.5
	0624300	*Providencia stuartii*	4.5
	3305573	*Enterobacter cloacae*	18.2
	1205000	*Ewingella Americana*	9.1
	1144572	*Escherichia coli*	4.5
	5304773	*Serratia fonticola*	9.1
	1205773	*Pantoea* spp	13.6
	1206773	*Serratia ficaria*	4.5
	0402000	*Shewanella putrefaciens*	9.1
	0306000	*Burkholderia cepacia*	4.5

*Frequency = adjusted to 1 decimal place.

Table 2. Antibiogram of the isolates from the chicken droppings.

Isolates	Antibiotics IZD (mm)								*MARI
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CTR	
Escherichia coli	0	0	16	0	12	0	0	25	0.750
K. pneumoniae	12	0	18	12	20	0	20	30	0.375
Burkholderia cepacian	0	0	13	0	21	0	0	25	0.625
Kluyvera intermedia	20	15	20	0	28	0	22	30	0.375

Continued

Enterobacter cloacae	0	0	0	0	0	0	0	0	1.000
Shewanella putrefaciens	0	0	11	0	20	0	0	11	0.750
Enterobacter aerogenes	20	15	0	25	20	0	15	18	0.500
Citrobacter braaki	0	10	20	0	18	19	21	25	0.375
Proteus mirabilis	18	12	15	28	25	0	0	25	0.500
Serratia odorifera	0	0	0	0	0	0	0	0	1.000
Pantoea spp	0	0	0	0	20	0	11	20	0.750
Acinetobacter baumannii	0	0	18	0	30	0	0	29	0.625
Proteus penneri	0	0	19	0	25	0	18	20	0.500
Providencia stuartii	20	20	12	15	28	0	10	30	0.500
Salmonella enteric spp *Arizona*	0	0	0	0	0	0	20	0	0.875
E. coli	20	13	0	22	15	0	15	20	0.625
K. pneumoniae	20	9	11	20	18	0	0	15	0.500
Burkholderia cepacian	16	15	19	24	21	0	25	22	0.250
Kluyvera intermedia	18	10	18	20	20	0	25	21	0.250
Enterobacter cloacae	17	0	14	13	20	0	0	20	0.625
Shewanella putrefaciens	25	15	0	20	20	0	12	20	0.375
Enterobacter aerogenes	20	15	18	22	20	0	19	21	0.250
Proteus mirabilis	26	0	20	30	30	0	20	30	0.250
Serratia odorifera	18	15	0	30	0	0	10	0	0.625
Pantoea spp	0	0	18	0	28	0	0	30	0.625
E. coli	20	17	0	30	20	0	22	25	0.250
K. pneumoniae	15	14	0	22	0	0	11	0	0.750
Burkholderia cepacian	0	0	18	20	30	0	25	30	0.375
Kluyvera intermedia	25	20	0	32	0	0	17	0	0.500
Enterobacter cloacae	13	0	12	22	0	0	12	0	0.875
Shewanella putrefaciens	15	0	20	12	10	0	15	20	0.750
Serratia odorifera	21	18	10	25	20	0	20	15	0.250
Pantoea spp	14	0	18	24	12	0	15	22	0.625
E. coli	22	12	0	27	18	0	10	20	0.500
K. pneumoniae	18	0	20	20	23	0	22	28	0.250
Serratia odorifera	20	0	15	0	20	0	0	27	0.625

KEY: OFL = Ofloxacin 5 μg; CAZ = Ceftazidime 30 μg; CRX = Cefuroxime = 30 μg; GEN = Gentamicin 10 μg; CTR = Ceftriaxone 30 μg; AUG = Augmentin 30 μg; CXM = Cefixime 5 μg; NIT = Nitrofurantoin 300 μg; ***MARI =** adjusted to 3 decimal places.

Table 3. Antibiogram of the isolates from the cow dungs.

Bacterial Isolates	Antibiotics IZD (mm)								*MARI
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CTR	
E. coli	15	10	20	15	28	0	18	35	0.500
K. pneumoniae	0	0	15	0	22	0	0	22	0.750
Enterobacter cloacae	20	20	20	25	30	0	20	30	0.875
Pantoea spp	0	0	18	0	30	0	0	30	0.625
Ewingella Americana	20	19	20	25	22	0	23	25	0.125
Serratia fonticola	20	17	0	28	21	0	20	27	0.250
Shewanella putrefaciens	0	0	0	0	0	0	0	0	1.000
Serratia ficaria	15	0	20	20	25	0	25	28	0.375
P. aeruginosa	0	0	12	0	20	0	0	30	0.750
Providencia stuartii	20	22	20	30	28	0	11	30	0.250
Burkholderia cepacian	0	0	0	0	21	0	0	25	0.750
K. pneumoniae	20	13	15	25	0	0	0	15	0.750
Enterobacter cloacae	19	13	20	15	20	0	17	20	0.375
Pantoea spp	0	0	0	0	25	13	0	20	0.750
Ewingella Americana	16	14	17	20	18	0	14	20	0.375
Serratia fonticola	20	20	0	25	21	0	17	23	0.250
Shewanella putrefaciens	20	0	20	25	10	0	20	0	0.500
K. pneumoniae	20	0	0	30	22	0	18	20	0.375
Enterobacter cloacae	15	0	20	12	10	0	15	20	0.750
Pantoea spp	20	13	0	30	17	0	20	25	0.375
K. pneumoniae	18	0	20	15	20	0	0	0	0.625
Enterobacter cloacae	17	15	20	17	20	0	15	20	0.375

KEY: OFL = Ofloxacin 5 μg; CAZ = Ceftazidime 30 μg; CRX = Cefuroxime = 30 μg; GEN = Gentamicin 10 μg; CTR = Ceftriaxone 30 μg; AUG = Augmentin 30 μg; CXM = Cefixime 5 μg; NIT = Nitrofurantoin 300 μg; ***MARI =** adjusted to 3 decimal places.

profile of the isolates with most of the isolates showing resistance to three or more antibiotics (above 0.2) hence are considered as multi-antibiotics resistant strains. Table 4 shows the results of the screening tests for AmpC production among the 16 MDR isolates. From the test, *Escherichia coli*, *Proteus penneri*, *Pantoea* spp, *Shewanella putrefaciens* were positive for AmpC production. The results of the screening tests for Metallo–β-lactamase (MBL) production (Table 5) among the 16 multidrug-resistant isolates revealed that *Serratia odorifera* and *Enterobacter cloacae* were positive for Metallo–β-lactamase (MBL) production. Table 6 shows the results of the screening tests for possible ESBL production

Table 4. AmpC production by the multi drug resistant isolates.

Enterobacteriaceae Isolates	CTXCX (mm)	CTX30 (mm)	RESULT
Acinetobacter baumannii	18	18	-
Pantoea spp	30	30	-
Salmonella enterica spp *Arizona*	30	30	-
Pantoea spp	22	20	-
Burkholderia cepacia	25	22	-
Serratia odorifera	22	16	-
Escherichia coli	30	25	+
Shewanella putrefaciens	25	23	-
Enterobacter cloacae	17	18	-
Proteus penneri	27	0	+
Burkholderia cepacia	25	25	-
Pantoea spp	22	18	-
Pseudomonas aeruginosa	27	28	-
Pantoea spp	22	12	+
Shewanella putrefaciens	20	15	+
Pantoea spp	25	28	-

Table 5. Metallo–B-lactamase (MBL) production by the multi drug resistant isolates.

SAMPLE CODE	MRPDP (mm)	MRP10 (mm)	RESULT
Acinetobacter baumannii	25	25	-
Pantoea spp	28	28	-
Salmonella enterica spp *Arizona*	30	30	-
Pantoea spp	28	30	-
Burkholderia cepacia	30	30	-
Serratia odorifera	22	0	+
Escherichia coli	26	30	-
Shewanella putrefaciens	35	32	-
Enterobacter cloacae	18	10	+
Proteus penneri	30	32	-
Burkholderia cepacia	30	30	-
Pantoea spp	28	30	-
Pseudomonas aeruginosa	30	30	-
Pantoea spp	22	18	-
Shewanella putrefaciens	18	15	-
Pantoea spp	30	28	-

Table 6. ESBL production by the multi-drug resistant isolates.

Enterobacteriaceae Isolates	Positive for ESBL	Negative for ESBL	Total
Acinetobacter baumannii	0	1	1
Pantoea spp	0	5	5
Salmonella enterica app Arizona	0	1	1
Burkholderia cepacia	0	1	1
Serratia odorifera	0	2	2
Escherichia coli	0	1	1
Shewanella putrefaciens	0	2	2
Enterobacter cloacae	0	1	1
Proteus penneri	0	1	1
Pseudomonas aeruginosa	0	1	1
Total	0	16	16

among the 16 multidrug-resistant isolates. The result showed that none of the isolates were positive for ESBL production.

4. Discussion

The nonstop incidence of antimicrobial resistance caused by selective pressure, continued abuse and misuse of antibiotics and the use of antibiotics in animal husbandry have created a very serious problem in the treatment of bacterial infections and has become a daunting task for public health practitioners worldwide since only a very few antibiotics are effective for use. The increasing emergence of multidrug resistance is making the issue more problematic.

In this study, we investigated the presence, identity and antimicrobial profile of Enterobacteriaceae isolated from chicken and cow dung in Onitsha, Anambra State, Nigeria. The results revealed the presence of forty three (69.4%) enterobacteriaceae. This demonstrates their dominance among the 62 gram negative isolates recovered. *E. coli* (15%) and *Klebsiella pneumoniae* (15%) were bacteriologically recovered from the chicken droppings swab samples as the most prevalent organisms which is in tandem with the report of Ejikeugwu *et al.* [14] who isolated similar organisms from anal swap samples from abattoir. However, *Klebsiella pneumoniae* and *Enterobacter cloacae* were the most prevalent Enterobacteriaceae isolated from cow dungs with a percentage of 18.2% each. Both bacteriological recoveries (chicken droppings and cow dungs) agree with the findings of Amador *et al.* [3] in Portugal who isolated various Enterobacteriaceae from Portuguese livestock manure. These bacteria are members of the Enterobacteriaceae family and part of the human normal flora.

The evaluation of the antimicrobial resistance profiles of the recovered isolates under study is paramount as antibiotic-resistant bacteria in animal excreta are

an emergent concern. The resistance profiles of all Enterobacteriaceae isolates were evaluated by exposure to eight antibiotics of four different classes for the phenotypic characterization of the isolates. These were chosen to represent the main antibacterial classes used in human medicine and livestock production in Nigeria. The resistance of all isolates from chicken dropping, except *Citrobacter braaki*, to Augmentin as well as the resistance of *Serratia odorifera* and *Enterobacter cloacae* to all the antibiotics tested can be attributed to the use of extended spectrum cephalosporins such as cefoxitin and cefotazim in livestock. The results of this research buttress the findings of [11] who reported that the presence of Carbapenemases producing Enterobacteriaceae in animals is becoming worrisome. These organisms, all of which are of public health importance, are in line with the report [2] who reported that Carbapenem-resistant Enterobacteriaceae are organisms of medical importance. As well as [18] in Southern China who recorded high level of Carbapenem-resistant *Acinetobacter* spp. from clinical infection and fecal survey samples in Portugal [3]. The antibiogram profiles of the bacterial isolates from both sites revealed that some of the bacterial isolates were highly resistant to commonly used agents although some of the isolates were sensitive to Ofloxacin and moderately sensitive to Ceftazidime. Most of the isolates were resistant to three or more antibiotic classes hence are considered as multi-antibiotics resistant strains. The results of isolates from chicken dropping show that only one isolate, *Providencia stuartii*, gave a MARI of <0.20 as it had a MARI of 0.13 with others having 0.20 and above while *Ewingella americana* and *Providencia stuartii* both had a MARI of 0.13 among organisms isolated from the cow dung. The multiple antibiotics resistance index (MARI) is a protocol used to explain the spread of bacteria resistance and resistant genes in any bacterial population [19] [20]. Generally, Multiple antibiotics resistance index above 0.20 means that bacteria isolates originating from such an environment has been exposed to indiscriminate use of several antibiotics in time past [19] [21]. The multi-drug resistance to the antibiotics of different classes observed in this study may be due to the increasing administration of quinolones to treat avian infections [3]. The unnecessary use of antibiotics for enhancement of growth and prevention of diseases in farm animals has impressed selective pressures that induce more resistance among bacteria in the community. From the sixteen isolates examined phenotypically for the production of metallo β-lactamase (MBL), two (12.5%) isolates were positive for the production of this enzyme. The production of MBL is this study is similar to a previous study conducted by Ejikeugwu *et al.* [12] in which MBL was detected in *Klebsiella* species isolated from cow anal swabs. Only two AmpC producing Enterobacteriaceae was detected when the isolates were phenotypically screened for the enzyme. This is similar to an earlier report by Ejikeugwu *et al.* [14] in which AmpC enzymes were significantly detected in the *E. coli* and *Klebsiella* species isolated from cow anal swabs from an abattoir in Abakaliki, Nigeria. These results illustrate that the AmpC and metallo β-lactamase (MBL) producing species isolated in this study are multidrug resistant. They also produce AmpC and metallo β-lactamase (MBL) en-

zymes which allow them to be resistant to the 2nd and 3rd generation cephalosporins which are clinically used to manage and treat serious bacterial infections. Furthermore, all the isolates screened phenotypically for ESBL production showed negative, this is in contrast to the review done by Madec *et al.* [11] where a study conducted among 699 *S. enterica* isolates from 1152 retail chickens reported a 24.6% rate of ESBL producers in Shanghai, China. This current study is relevant and a springboard to the increasing prevalence of antibiotic resistance in the non-nosocomial environment such as abattoir. More so, it provides acceptance to the possible abuse and irrational use of antibiotics in animal husbandry and for other non-clinical purposes. Hitherto, fecal excrement of chicken and cows in constant contact with humans who are husbandry rearers, pose high risk of cross contamination and further affects the antimicrobial resistance status and ultimately, the public health standards of nations.

Study Limitations: The molecular characterization of the isolates to further confirm the identity of the isolates wasn't conducted at the time of this writing due to limited funding. Also, the genes responsible for drug resistance could not be identified for the same financial constraints.

5. Conclusions

The bacteria of public health importance isolated from these sites and their antibiogram profile have shown the need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health as well as contribute to the fight against antibiotic resistance.

What is known about this topic?

World over:

- Resistance of the Enterobacteriaceae to commonly used antibiotics in the last decade has been of an alarming proportion, causing increased public health concerns.
- The last few years have witnessed the proliferation of several extended-spectrum β-lactamases (ESBL) and AmpC-producing Enterobacteriaceae (EPE) in both human and animal health management globally.
- Animals have been touted as the transmission link of ESBLs/AmpCs for humans and demanded urgent response.

What this study adds

This study has showed that:

- There is the presence of resistant strains among *enterobacteriaceae* found in chicken and cow droppings.
- The need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health.
- More evidence that animals could serve as the transmission link of ESBLs/AmpCs in humans.

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Conflicts of Interest

None to declare.

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Authors' Contributions

CCA initiated the concept of the research, performed the Lab work and wrote the first draft. ANO elaborated the idea, designed the work and as well as supervised the lab work. PAE and MUO helped with laboratory works while JK participated in writing the manuscript. All the authors have read and agreed to the final manuscript.

References

[1] Almugadam, B.S., Osman, N.A., Alaaeldeen, B.A., Ahmed, B.E. and Wang, L. (2018) Prevalence and Antibiotics Susceptibility Patterns of Carbapenem Resistant *Enterobacteriaceae*. *Journal of Bacteriology and Mycology*, **6**, 187-190. https://doi.org/10.15406/jbmoa.2018.06.00201

[2] Oli, A.N., Itumo, C.J., Okam, P.C., Ezebialu, I.U., Okeke, K.N., Ifezulike, C.C., Ezeobi, I., Emechebe, G.O., Okezie, U.M., Adejumo, S.A. and Okoyeh, J.N. (2019) Carbapenem-Resistant *Enterobacteriaceae* Posing a Dilemma in Effective Healthcare Delivery. *Antibiotics*, **8**, Article No. 156. https://doi.org/10.3390/antibiotics8040156

[3] Amador, P., Fernandes, R., Prudêncio, C. and Duarte, I. (2019) Prevalence of Antibiotic Resistance Genes in Multidrug-Resistant *Enterobacteriaceae* on Portuguese Livestock Manure. *Antibiotics*, **8**, Article No. 23. https://doi.org/10.3390/antibiotics8010023

[4] Van den, H.M.S., Gouws, P.A. and Hoffman, LC. (2018) Importance and Implications of Antibiotic Resistance Development in Livestock and Wildlife Farming in South Africa: A Review. *South African Journal of Animal Science*, **48**, 401-412. https://doi.org/10.4314/sajas.v48i3.1

[5] Moravej, H., Zahra, M., Maryam, Y., Mohammad, H., Ali, M., Mehrdad, M.M. and Reza, M. (2018) Antimicrobial Peptides: Features, Action, and Their Resistance Mechanisms in Bacteria. *Microbial Drug Resistance*, **24**, 747-767. https://doi.org/10.1089/mdr.2017.0392

[6] Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A. and Nautiyal, A. (2017) Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. *Plants*, **6**, Article No. 16. https://doi.org/10.3390/plants6020016

[7] Oli, A.N., Eze, D.E., Gugu, T.H., Ezeobi, I., Maduagwu, U.N. and Ihekwereme, C.P. Multi-Antibiotic Resistant Extended-Spectrum Beta-Lactamase Producing Bacteria Pose a Challenge to the Effective Treatment of Wound and Skin Infections. *The Pan*

African Medical Journal, **27**, Article No. 66.

[8] Zhang, R., Liu, L., Zhou, H., Waichi, E., Li, J., Fang, Y., Li, Y., Liao, K. and Chen, S. Nationwide Surveillance of Clinical Carbapenem-Resistant *Enterobacteriaceae* (CRE) Strains in China. *EBioMedicine*, **19**, 98-106. https://doi.org/10.1016/j.ebiom.2017.04.032

[9] Ali, M., Amal, J., Mohammed, A., Wael, M., Merin, G., Sadananda, A., Sanjay, R., Darshan, D.D., Chitra, J., Sajith, V., Aftab, A.K., Jilani, S. and Poojdev, J. (2020) Antimicrobial Resistance, Mechanisms and Its Clinical Significance. *Disease-A-Month*, **66**, Article ID: 100971

[10] Hosuru Subramanya, S., Bairy, I., Nayak, N., Amberpet, R., Padukone, S., Metok, Y., Bhatta, D.R. and Sathian, B. (2020) Detection and Characterization of ESBL-Producing *Enterobacteriaceae* from the Gut of Healthy Chickens, *Gallus gallus domesticus* in Rural Nepal: Dominance of CTX-M-15-Non-ST131 *Escherichia coli* Clones. *PLoS ONE*, **15**, e0227725. https://doi.org/10.1371/journal.pone.0227725

[11] Madec, J., Haenni, M., Nordmann, P. and Poirel, L. (2017) Extended-Spectrum *β*-Lactamase/AmpC- and Carbapenemase-Producing *Enterobacteriaceae* in Animals: A Threat for Humans? *Clinical Microbiology and Infection*, **23**, 826-833. https://doi.org/10.1016/j.cmi.2017.01.013

[12] Ejikeugwu, C., Duru, C., Eluu, S., Oguejiofor, B., Ezeador, C., Ogene, L. and Iroha, I. (2017) Isolation and Phenotypic Detection of Metallo-Beta-Lactamase (MBL)-Producing Klebsiella Species from Cow Anal Swabs. *Global Journal of Pharmacy & Pharmaceutical Sciences*, **2**, 54-58. https://doi.org/10.19080/GJPPS.2017.02.555586

[13] Hansen, F., Hammerum, A.M., Skov, R.L., Giske, C.G., Sundsfjord, A. and Samuelsen, O. (2012) Evaluation of Rosco Neo-Sensitabs for Phenotypic Detection and Subgrouping of ESBL-, AmpC- and Carbapenemase-Producing *Enterobacteriaceae*. *APMIS*, **120**, 724-732. https://doi.org/10.1111/j.1600-0463.2012.02898.x

[14] Ejikeugwu, C., Nworie, O., Agah, M.V., Oguejiofor, B., Ovia, K., Nworie, C.O., Iwunze, A.C., Nwambeke, A. and Edeh, C. (2018) Bacteriological and Antibiogram of AmpC Producing *Enterobacteriaceae* Isolated from Abattoir. *Microbiology: Current Research*, **2**, 37-41. http://www.alliedacademies.org/microbiology-current-research/

[15] Federal Republic of Nigeria (2010) 2006 Population and Housing Census: Priority Table, Volume III. National Population Commission: Population Distribution by Sex, State, LGA & Senatorial District, Abuja.

[16] Laine, L., Perry, J.D., Lee, J., Oliver, M., James, A.L., De La Foata, C., Halimi, D., Orenga, S., Galloway, A. and Gould, F.K. (2009) A Novel Chromogenic Medium for Isolation of *Pseudomonas aeruginosa* from the Sputa of Cystic Fibrosis Patients. *Journal of Cystic Fibrosis*, **8**, 143-149. https://doi.org/10.1016/j.jcf.2008.11.003

[17] Clinical and Laboratory Standards Institute (CLSI) (2017) Performance Standards for Antimicrobial Disk Susceptibility Tests. 12th Edition, Clinical and Laboratory Standards Institute, Wayne, PA.

[18] Li, S., Duan, X., Peng, Y. and Rui, Y. (2019) Molecular Characteristics of Carbapenem-Resistant *Acinetobacter* spp. from Clinical Infection Samples and Fecal Survey Samples in Southern China. *BMC Infectious Diseases*, **19**, Article No. 900. https://doi.org/10.1186/s12879-019-4423-3

[19] Ejiofor, O.S., Ajunwa, O.M., Ezeudu, C.E., Emechebe, G.O., Okeke, K.N., Ifezulike, C.C., Ekejindu, I.M., Okoyeh, J.N., Osuala, E.O. and Oli, A.N. (2018) The Bacteriology and Its Virulence Factors in Neonatal Infections: Threats to Child Survival Strategies. *Journal of Pathogens*, **2018**, Article ID: 4801247.

https://doi.org/10.1155/2018/4801247

[20] Hosu, M.C., Vasaikar, S., Okuthe, G.E. and Apalata, T. (2021) Molecular Detection of Antibiotic-Resistant Genes in Pseudomonas aeruginosa from Nonclinical Environment: Public Health Implications in Mthatha, Eastern Cape Province. *South Africa. International Journal of Microbiology*, **2021**, Article ID: 8861074. https://doi.org/10.21203/rs.3.rs-31330/v1

[21] Ejiofor, S.O., Edeh, A.D., Ezeudu, C.E., Gugu, T.H. and Oli, A.N. (2016) Multi-Drug Resistant Acute Otitis Media amongst Children Attending Out-Patient Clinic in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, South-East Nigeria. *Advances in Microbiology*, **6**, 495-501. http://www.scirp.org/journal/aim https://doi.org/10.4236/aim.2016.67049

Appendix

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No
Title and abstract	1	1) Indicate the study's design with a commonly used term in the title or the abstract	1
		2) Provide in the abstract an informative and balanced summary of what was done and what was found	1
		Introduction	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
		Methods	
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	1) Give the eligibility criteria, and the sources and methods of selection of participants	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	NA
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	NA
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	1) Describe all statistical methods, including those used to control for confounding	NA
		2) Describe any methods used to examine subgroups and interactions	NA
		3) Explain how missing data were addressed	NA
		4) If applicable, describe analytical methods taking account of sampling strategy	NA
		5) Describe any sensitivity analyses	NA
		Results	
Participants	13*	1) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	NA
		2) Give reasons for non-participation at each stage	NA
		3) Consider use of a flow diagram	NA

Continued

Descriptive data	14*	1) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	NA
		2) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	6
Main results	16	1) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		2) Report category boundaries when continuous variables were categorized	12
		3) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	12
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	12
		Discussion	
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalizability	21	Discuss the generalizability (external validity) of the study results	NA
		Other information	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

NA: Not applicable. *Give information separately for exposed and unexposed groups. **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org/.

List of Abbreviations

MARI: Multiple Antibiotics Resistance Index
MBL: Metallo-β-lactamase
API: Analytical Profile Index
CRE: Carbapenem-resistant Enterobacteriaceae
MAC: MacConkey Agar
SSA: Salmonella-Shigella Agar
EMB: Eosin Methylene Blue Agar
GNB: Gram Negative Bacteria