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# Prevalence And Molecular Charaterization Of Multiple Resistance Genes Associated With Escherichia Coli Isolated From Poultry Farms In Abakaliki Metropolis Ebonyi State.

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***Abstract***
*This study was conducted to determine the prevalence of multiple resistance genes associated with Escherichia coli from poultry farms in Abakaliki me-tropolis, Ebonyi State, Nigeria. A total of 500 poultry samples were collected from poultry samples as follows: cloacal n=125, feed n=125, water n=125 and meat n=125 and analyzed in the Microbiology Laboratory Complex of Ebonyi State University using microbiological standard. The E. coli isolates were identified by morphology, culture and biochemical tests and further subjected to antimicrobial susceptibility testing against different classes of antibiotics using Kirby Bauer disk diffusion method. The multiple antibiotics resistance index was determined and thereafter molecular profiling was carried out to determine the gene mediating multiple drug resistance in the isolates. From the results, out of 500 samples analyzed 225 of the samples were positive for E. coli while others were negative. The overall prevalence of E. coli isolates from the poultry farm were 90 (72.0 %) from cloaca swab, 75(60.0 %) from feed sample, 86(68.8.0 %) from drinking water and 67(53.6 %) among meat swab. The antibiotic susceptible and resistance test on the E. coli isolates yielded high resistance of 100 % to gentamycin, erythromycin, and nitrofur-antoin from clocoal swabs, followed by drinking water where the E. coli isolates were 78 % susceptible to cefepime and colistin sulfate. However, in meat swabs and feed sample most of the E. coli were found to be susceptible to the antibiotics were up to 50 % with low inhibition zone diameter. Multiple antibiotics resistance index (MARI) of the isolates ranged from 0.58 - 0.65 which showed that the isolates are multidrug resistant organism. Agarose gel electrophoresis using 50bp showed in this study that 80 % of the isolates had the dfrA1, blaTEM, tet(A) and qnrA as the most commonly detectable resistant markers. The result also revealed that dfrA, blaTEM and qnrA was the most common resistant gene among the E. coli isolates analysed. The resistance of these isolates (E. coli) to the commonly used antibiotics substantiates the reports that E. coli from poultry origin habours multidrug resistant genes and the significant contribution of poultry farming to the spread of antimicrobial resistance, with potential consequences for both farmers and human health.*

***Keywords:*** *Antimicrobial resistance markers, poultry, bacterial resistance, Antibacterial susceptibility, Inhibition zones.*

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## I. Introduction

The term "Poultry" is used to describe any kind of domesticated birds raised for its utility, and traditionally the word has been used to refer to wildfowl (Galliformes and waterfowl, Anseriformes) (Chadd *et al.,* 2016) " Poultry is a good source of high-quality animal protein with a good health benefits for the growing population which provides food and income (Abd el-aziz *et al.,* 2021). Poultry meat and eggs provide nutritionally beneficial protein of high quality, this is accompanied by low levels of fat which have a favorable mix of fatty acids (Aarestrup *et al.,* 2013). Chicken meat contains about two to three times as much polyunsaturated fats as most types of red meat when measured by weight (Abbasi-ghozi et al., 2015). There are two distinct models of poultry production; the broiler mainly for meat productions and layers which is for eggs production. Meanwhile poultry products have been reported to be associated reservoir of multidrug resistance genes globally (Abdallah , 2011), due to continuous increase in the consumption of poultry products leading to the use available human

antibiotics for poultry prophylaxis, infection treatment and growth promotions which results in more AMR emergence globally (Brower et al., 2017).

This alarming increase in the rate of antibiotics resistance, multidrug resistance genes emergence, failure of chemotherapeutic agents and abuse in antibiotics usage has poised a significant challenge to public health. More so, there is a notable high prevalence of MDR genes in poultry products which poses a critical damage to public health but has remain poorly understood, thus hindering its effective control.

The encumbrance effects of MDRGs to public health in Abakaliki includes; difficult to treat inf-ections, more expensive antibiotics, alternative medication which prove more toxic to host, delay of effective treatment, incurable diseases (Brown and Mc Cue, 2022). Others includes; increased human sufferings and illnesses, organ disabilities, increased prevalence of food contamination and food poison by microorganisms and organ failure that cause million deaths annually in Abakaliki (Carraso et al., 2022). Moreover, Reports has it that E. coli infections in both animals and human are becoming increasingly incurable or too difficult to treat due to the rise in AMR thus causing a significant challenge to human and animal health (Chadd et al., 2016). E. coli are also associated with many animals and human including; cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), traveler's diarrhea, pneumonia, and neonatal meningitis (Sallam et al., 2017). Escherichia coli is distributed among poultry of all ages. The Escherichia bacteria is a natural inhabitant of the poultry gut and most other animals. Normally, it is kept in check by other bacteria in the gut, but during complications it can leads to severe discomfort, illness, and mortality. E. coli is opportunistic in nature and grow rapidly in times of stress. In poultry houses, E. coli, commonly referred to as colibacillosis, is spread through water, feed and fecal contamination. Colibacillosis infections can become a problem due to poor managements, i.e if birds are not allowed regular access to clean fresh feed and water, and if litters are allowed to remain wet due to poor ventilation, the bacteria will spread rapidly through fecal contamination and respiratory mucosa.

## II. Materials And Methods

Samples Collections and processing

The samples were aseptically and randomly collected from different poultry origin as fellows; poultry birds cloaca and meat (125) each using sterile swab sticks to swab the cloaca and meat surface in a zigzag motion, drinking water (125) through the use of well labeled sterile syringe and container, poultry feeds (125) using a well labeled aseptic spatula and container into a clean zip lock bags in the morning hours and transported within 1h of collection to the Microbiology laboratory complex of Ebonyi State University Abakaliki, for bacteriological analysis.

Bacteriological Analysis.

The collected samples were inoculated into aseptically prepared nutrient broth and incubated at 35O C for 18-24 h. After incubation all the tubes showing turbidity were bacteriologically cultured on nutrient agar, Eosin methylene Blue (EMB), Macconkey agar Agar (Oxoid UK) and incubated at 35 OC for 18-24 h for selective isolation of typical E.coli growth (red and pink colonies). The enumeration of the microbial colonies was by pour plate techniques while colonies were counted using colony counter and result expressed as cfu/g.

The microbial colonies identification was achieved based on morphological characteristics and biochemical tests (Cheesebrough, 2016). Each plate was visually examined for the presence of colonies that resembles E. coli and suspected colonies were aseptically sub cultured into a fresh prepared nutrient agar plates for purification.

Antimicrobial Susceptibility Test

The isolates were screened for antimicrobial susceptibility and resistance using the Kirby-Bauer agar disk diffusion method of (CLSI, 2015). Single antibiotics antibiotics disks including Gentamycins (CN) (10 µg), ciprofloxacin (CIP) (5 µg), trimethoprim (TM) (5 µg), nitrofurantoin (F) 300 µg), cefepime (FEP) (30 µg), ceftriaxone(CRO)(10 µg), erythromycins (E) (15 µg), cefpodoxime (PX) (10 µg), meropenem (MRP) 10 µg), tetracyclin (TE) (30 µg), chrophenicol (CH) (10 µg) and colistin sulphate (CS) (10 µg) (Abruzzi, Italy) were used for the antibiogram.

The test isolates was prepared to match 0.5 McFarland turbidity standards in order to standardize the inoculums, and then aseptically swabbed on the surface of Mueller-Hinton agar (MH) plate using forceps and incubated 35° C for 18- 24 h. The inhibition zones diameter (IZD) were measured with meter rule and interprete as susceptibility or resistance according to the CLSI (2015) guidelines.

Determination of MAR Index Determination of MAR index was done using the formula; MAR Index = a/b, where a is the number of antibiotics an isolate is resistant and (b) is the total number of the antibiotics used in the study (b) as described by Moses et al., (2020).

## III. Results

The morphological and biochemical characteristics of Escherichia coli isolates from poultry farms sample indicates that the isolates were rod shape, smooth, pink and somewhat shining, negative to Gram stain, urease and oxidase test but positive to motility, lactose, indole, coagulase and catalase test (Table 1).

**Table 1**: Morphology and Biochemical characteristics of bacteria Isolates

| GS | S | C | Ca | TSI | Ox | Mo | Vp | Mr | I | C | GL | L | Ur | Co | Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| - | Rod | pink | | A/G | - | + | - | + | + | - | + | + | - | + | E. coli |

KEYS: A/A= Acid/Alkaline, A=Acid,A/G-Acid/Gas, VP=Voges-Proskauer, (+)=positive, (-)= negative, SSA-*Salmonella* /*Shigella* agar

The overall prevalence of *E. coli* isolates from the poultry farm was 90(72.0 %) from cloacal swab, 75(60.0 %) from feed sample, 86(68.8.0 %) from drinking water and 67(53.6 %) among meat swab

(Table 2).

| Samples | Nos of samples collected | Prevalence (%) |
|---|---|---|
| Cloacal swab | 125 | 90(72.0) |
| Feeds | 125 | 75(60.0) |
| Drinking water | 125 | 86(68.8) |
| Meats | 125 | 67(53.6) |
| Total | 500 | |

However, the *E. coli* isolates showed high level of resistance to gentamycin, nitrofurantoin and erythromycin ranging from 60 % to 100 % among the isolates from cloacal swabs and feed samples. Average resistance was observed among the isolates to Ceftriaxone and Ciprofloxacin. All the *E. coli* isolates reported in this study were 20% to 50% susceptible to chloramphenicol and tetracycline.

**Table 3**: Antibiotic Susceptibility and Resistance Pattern of *E. coli* Isolated from Poultry Cloacal Swab and Feed Samples

| | Cloacal Swab (n= 15) | | Feed (n=20) | |
|---|---|---|---|---|
| Antibiotics (µg) | R (%) | S (%) | R (%) | S (%) |
| Gentamycin (10) | 15 (100) | 0 (00) | 20 (100) | 0(00) |
| Nitrofurantoin (300) | 15(100) | 0(00) | 20 (100) | 0(00) |
| Erythromycin (15) | 15 (100) | 0(00) | 20 (100) | 0(00) |
| Trimethoprim (5) | 9 (60) | 7(46.6) | 14 (70) | 6(30) |
| Cefepime (30) | 10 (66.7) | 5(33.3) | 16(80) | 4(20) |
| Meropenem (10) | 10(66.7) | 5(33.3) | 16 (80) | 4(20) |
| Colistin sulfate(10) | 9 (60) | 6 (40) | 16 (80) | 4 (20) |
| Ceftriaxone (30) | 11 (73.3) | 4 (26.7) | 15 (75) | 5 (25) |
| Ciprofloxacin (5) | 12(80) | 3(20) | 12 (80) | 6(30) |
| Cefopodoxime (10) | 10(66.7) | 5 (33.3) | 13(65) | 7 (35) |
| Chloramphenicol (10) | 11(55) | 9(45) | 11(55) | 9(45) |
| Tetracycline (10) | 12(80) | 6(30) | 14(70) | 6(30) |

**Table 4**: Antibiotic Susceptibility and Resistance Pattern of *E. coli* Isolated from Samples of Poultry Drinking Water.

| | Drinking water | | meat (n=9) | |
|---|---|---|---|---|
| Antibiotics (µg) | R (%) | S (%) | R (%) | S (%) |
| Gentamycin (10) | 6(66.6) | 3(33.3) | 5(50) | 5 (50) |
| Nitrofurantoin (300) | 6 (66.6) | 3 (33.3) | 6(60) | 4 (40) |
| Erythromycin (15) | 5 (55.5) | 4 (44.4) | 7 (70) | 6(60) |
| Trimethoprim (5) | 4(44.4) | 5(55.5) | 4(40) | 3 (30) |
| Cefepime (30) | 2(22.2) | 7 (77.7) | 5 (50) | 5(50) |
| Meropenem (10) | 3(33.3) | 6 (66.6) | 6 (60) | 4(40) |
| Colistin sulfate(10) | 4(44.4) | 5 (55.5) | 6 (60) | 4(40) |
| Ceftriaxone (30) | 5 (55.5) | 4 (44.4) | 4 (40) | 6 (60) |
| Ciprofloxacin (5) | 5(55.5) | 4 (44.4) | 5 (50) | 5 (19.2) |
| Cefopodoxime (10) | 3 (33.3) | 6 (66.6) | 4 (40) | 6 (60) |
| Chloramphenicol (10) | 6(66.6) | 3(33.3) | 7(70.0) | 3(30.0) |
| Tetracycline (10) | 4(44.4) | 5(55.5) | 6(60) | 4(40) |

Key: N = Number tested, S = Susceptible. R = Resistance, % = Percent.

Multiple antibiotic resistance was reported among 25 % of the *E. coli* isolates from the poultry birds within the farms at Abakaliki. Multiple antibiotics resistance index (MARI) of the isolates ranged from 0.58 - 0.65.

**Table 5:** Multiple Antibiotics Resistance Index (MARI) of *E. coli* Isolated from Poultry Farms Sample.

| | MARI of *E. coli isolates* |
|---|---|
| Water | 0.65 |
| Meat | 0.62 |
| Cloacal | 0.58 |
| Feed | 0.64 |
| Average MARI | 0.62 |

Key = *E. coli – Escherichia coli,* and MARI = multiple antibiotics resistance index

Agarose gel electrophoresis using 50bp showed in this study that 80 % of the isolates had the dfrA1, blaTEM, tet(A) and qnrA as the most commonly detectable resistant markers. The result also revealed that dfrA, blaTEM and qnrA was the most common resistant gene among the *E. coli* isolates analyzed. The resistance of these isolates *(E. coli*) in this study to the commonly used antibiotics substantiates the reports that *E. coli* from poultry origin habours multidrug resistant genes and the significant contribution of poultry farming to the spread of antimicrobial resistance, with potential consequences for both farmers and human health.

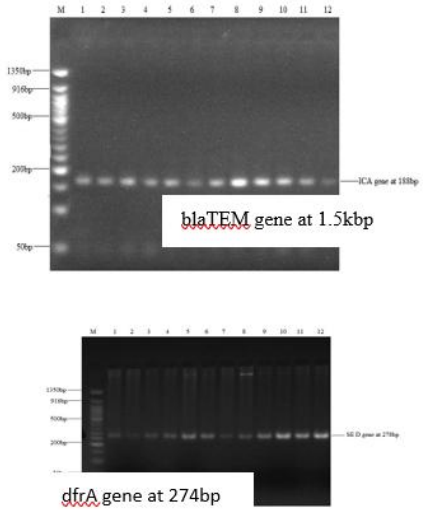


**Plate 1 & 2:** Gel image showing the amplification of blaTEM gene at 1500bp dfrA gene at 274bp. A 50bp DNA ladder was used to estimate the base pair size of the amplicons. Lane M =50bp molecular marker.

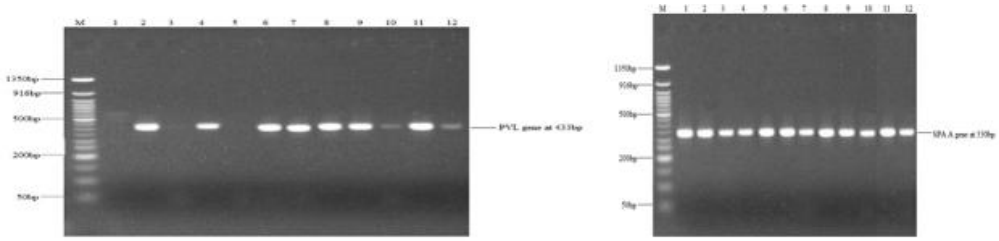

Plate 3& 4: Gel image showing the amplification of tetA gene at 433bp and catA gene at 186bp. A 50bp DNA ladder was used to estimate the base pair size of the amplicons. Lane M =50bp molecular marker.

## IV. Discussion

Poultry is one of the reservoir of multidrug resistance genes in organism like *E. coli* the leading cause of opportunistic infections.

The rate of antibiotics resistance, multidrug resistance genes emergence by microorganism and increasing failure of chemotherapeutic agents in our society today is alarming globally, this is attributed to the repercussion of ongoing overuse of antimicrobial agents for treatment, prophylaxis, and metephylaxis of animals in livestock keeping as most of it belong to similar classes of human drugs with broad spectrum of activities.

This study prevalence and molecular charaterization of multiple resistant genes associated with E. coli isolated from poultry farms showed relative high microbial counts, an indicative of high level of microbial

contamination of poultry farms and poultry farm products within Abakaliki metropolis which ranged from 3.1×105 cfu/ML to 8.0×105 cfu/mL. Out of 500 samples analyzed, 225 of the samples were positive for E. coli while others are negative. The overall prevalence of E. coli isolates from the poultry farm was 90(72.0 %) from cloacal swab, 75(60.0 %) from feed sample, 86(68.8.0 %) from drinking water and 67(53.6 %) among meat swab (Table 2) The results of antibiotics susceptibility patterns of E. coli isolated from poultry cloacal swab and feed samples were shown in Table 2. the E. coli were completely resistance (100 %) to gentamycin, nitrofurantoin, erythromycin followed by ciprofloxacin (80 %), ceftriaxone (73.3 %), tetracycline (70 %), cefpodoxime, cefepime and meropenem (66.7 %) and closely followed by trimethoprim and colistin sulphate (60 %) and chloramphenicol (55 %) in cloacal swab

Meanwhile E. coli isolates from feed samples were 80 % - 50 % resistance to all the antibiotics used including gentamycin, nitrofurantoin and erythromycin. this high level antibiotic resistance resistance could be attributed to uncontrolled use of antibiotics as antibiotic growth promoter.

In drinking water samples and meat swab E.coli isolates showed high resistance of 6(66.6 %) to gentamycin, chlorophencol and nitrofurantoin, erythromycin, ceftriaxone and ciprofloxacin 5(55.5 %), trimethoprim 4(44.4 %), cefepime 2(22.2 %), meropenem 3(33.3 %), colistin sulphate 4(44.4 %) and cefpodoxime 3(33.3 %), tetracycline 4(44.4 %) and 2(22.2 %) respectively. Multiple Antibiotics Resistance Index (MARI) multiple drug resistance E. coli was detected and the isolates had MARI of 0.65 in water samples, 0.62 in meat samples, 0.58 in cloacal samples and 0.64 in feed samples. Acording to previous studies organisms with MARI above 0.2 potentially posses antibiotic resistance genes.

Agrose gel electro genes for multi- drug resistance. Out of the 12 molecularly analysed isolates, all the isolates habours multidrug resistance gene. All PCR products amplified were of the sizes expected per genetic marker of resistance except tet(A) which showed a product twice the expected size (plate 1- 4). In all, the most frequently detected resistance gene was dfrA for trimethoprim (74.37 %) followed by blaTEM for β-lactam (73.75 %), for tetracycline tetA and tetB (73.13 %) and catA1, either one or both together for chloramphenicol.

The least detected resistance genes were qnrA for ciprofloxacin and ere(A) for erythromycin (0.63 %). The representative members of antibiotic classes used in this study showed that *E. coli* isolates resistant to meropenem harboured the blaTEM gene, and none had the blaCTX-M-1 gene (Figure 1 - 4). Moreover, in this study, all isolates resistant to tetracycline had either the tet(A) or tet(B) resistant marker. Further, our results showed that these resistant genes do occur together more commonly than they do singly. Thus these multi- drug resistance of greater proportion of the isolates that could pose a public health threat to the populace in Abakaliki metropolis and demand quick intervention.

## V. Conclusion

This study has shown that poultry farm in Abakaliki were contaminated with multiple drug resistance bacteria and a widespread incidence of the antimicrobial resistance and multidrug resistance gene of the isolates to the antibiotics commonly used in poultry farming, which could pose a negative effects on public health in Abakaliki. The results of the resistance profile suggest that there are certain patterns that reflect the unregulated use and misuse of antibiotics in poultry, with those antibiotics that are used less frequently, such as meropenem, cefopodoxime and trimethoprim showing low resistance prevalence. Finally MDRGS is a critical human and animal health issue globally, resulting in progressive loss of antimicrobials efficacy in the face of increasing abundance of exposure to resistant bacteria, with no or fewer new classes of antimicrobials being developed to fill the gaps.

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