# Antibiotic Resistance and Plasmid Profile of *Escherichia Coli* from Feacal Samples of Cattle From Abattoir In Keffi Metropolis, Nasarawa State Nigeria

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***Abstract-*** Antimicrobial use in animal's production has led to the emergence of resistant of *Escherichia coli*. A study on antibiotic resistance and plasmid profile of *E. coli* from faecal samples of Cattle from Keffi Abattoir, Nigeria was carried out. A total of 50 faecal samples were collected and *E. coli* was isolated and identified using standard microbiological method. The antibiotic resistance was determined in complianc with the clinical and laboratory standard institute (CLSI) method while the plasmid DNA of the multiple antibiotic resistance isolates were determined using stardand molecular method. 50 isolates of E.coli were obtained from the 50 faecal samples and the isolates were more resistance to nalidixic acid (98.0%), cefotaxime (96.0%), ceftazidine (82.0%) but less resistance to gentamicine (8.0%), amoxycilline/clavulanic acid (12.0%) and impinem (14.0%).The most common multiple antibiotic resistance (MAR) index was 0.6 with a percentage occurrence of 16.0%. while the most common antibiotic resistance phenotype of the isolates were NA-S-CRO-CTX-CAZ-SXT and CRO-CTX-SXT-CN-AMC-CIP-IPM-S-CAZ with a resistance of 12%. The isolates had 1 plasmid each with an estimated molecular size of 1200bp. Further studies on molecular diversity of the resistance isolates should be carried out, there is need for further awareness and education on abuse or excess used of antibiotics in animal farms and veterinary medicine. susceptibility surveillance.

***Index Terms***- Antibiotic, Resistance, *Escherichia coli*, Plasmid, Abattoir.

## I. Introduction

*Escherichia coli* is one of the common microbial flora of gastrointestinal tract of human and animals but may become pathogenic to both (Muhammad *et al.,* 2009). *Escherichia coli* are also the most frequently encountered microorganism in the food industry (Obeng *et al*., 2012). Diseases caused by these pathogens range from bloody diarrhea, enteritis, hemorrhagic colitis (HC), hemolyticuremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Nataro and Kaper, 1998). The abattoir environment plays important role in the meat industry and is an important component of livestock industry in Nigeria, (Lawan *et al*., 2013) Abattoirs pollute the environment either directly or indirectly from their various processes (Bello *et al, 2008*). The numerous wastes produced by abattoir operation not only pose a significant challenge to effective environmental management but also are associated with decrease air quality of the environment, potential transferable antimicrobial resistance patterns, and several infectious agents that can be pathogenic to human. (Nwanta *et al.,* 2008). Published studies have documented a variety of contaminants, microbial agents and health effects in those occupationally or accidentally exposed to improperly managed abattoir waste (Adelegan, 2002; Adeyemo, 2002; Abiade *et al* 2006). Animal feces are potential source of antibiotic resistant bacteria, if released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to human health (Roy *et al*., 2009). This study aims at determining antibiotic resistance and plasmid profile of *Escherichia coli* isolated from faecal samples of cattle in Keffi Abattoir, Nasarawa State, Nigeria.

## II. MATERIALS AND METHODS

### 2.1 Study area

The study area was Keffi metropolis, Nasarawa State. Keffi is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the Capital of Nasarawa state Keffi is located between latitude 8°5 N of the equator and longitude 7°8 E and situated on an altitude of 850m above sea level (Akwa *et al*., 2007).

### 2.2 Sample Collection

A total of fifty (50) samples were collected from Abattoir in Keffi metropolis Nigeria using sterile container and transported to Microbiology Laboratory, Nasarawa State University, Keffi, Nigeria for analysis.

### 2.3 Isolation of *Escherichia coli*

The isolation of *Escherichia coli* was carried out as described by Opere *et al*. (2013) with some modifications; the fecal samples were aseptically cultured on Eosin Methylene Blue (EMB) Agar plates by streaking, using sterile wire loop and incubated at $37^0$c in the incubator for 24 hours. The plates were observed after 24 hours incubation, colonies with greenish metallic sheen indicates presumptively the presence of *Escherichia coli*; further biochemical and immunological tests were performed to confirm the organism.

### 2.4 Identification of *Escherichia coli*.

The cultural, morphological and biochemical characteristic of the respective isolates were compared with the criteria in

Bergey's manual of Determinative Bacteriology (1994). The biochemical test used in the identification and characterization of the isolates include: gram staining, indole production, methyl red –Voges Proskauer and Citrate utilization, test.

**2.5 commercial kit (KB003H125$^{TM}$ ) Identification of *Escherichia coli***

The suspected *E. coli* isolates that were Gram negative, rod shape, idole positive, methyl red positive, citrate and voges-proskauer negative were further identified using KB003H125$^{TM}$ kits following the manufacturer instructions as follows; 2-3 pure colonies of suspected organism was transferred 5ml of sterile normal saline and the turbidity of the suspension was adjusted to the turbidity equivalent to mcferland 0.5. The kits was aseptically open by sealing off the sealing foil and 50µl of the subject inoculums was inoculated in each well of the kit and the kit was seal off using the sealing foil and incubated at 37$^{o}$C for 18h. after 18h incubation 2-3 drops of reagent R036 and 1 drop of R015 was added into the well 6, 2-3 drops of R0029 and 1 drops of R030 was added to well 9; 1-2 drops of 1007 reagent was added to well 10 and finally 1-2 drops of R008 was added to well 11. The results was read and interpreted as per the standard given in the identification index.

**2.6 Antibiotics Susceptibility Testing**

The antibiotics susceptibility test of the isolates was carried out using Kirby-Baeur disk diffusion method with some modifications as described in Clinical Laboratory Standard Institute manual (CLSI, 2012). The antibiotic disks were firmly placed on the sterile Muiller Hinton Agar (MHA) plates seeded with test organisms, standardized to 0.5 MacFarland's standard (equivalent to 10$^{5}$Cfu) and incubated at 37$^{o}$C for 24 hours. Diameter of zones of inhibition was then measured to the nearest millimeter and reported in accordance with the antimicrobial susceptibility breakpoint.

**2.7 Determination of Multiple Antibiotic Resistance (MAR) Index**

**Determination of Multiple Antibiotic Resistance (MAR) Index**

The MAR index was determined for all isolates resistant to at least two of the antibiotics tested as follows (Ngwai *et al.*, 2014):

MAR Index = No. of antibiotics isolate is resistant to
No. of antibiotics isolate is exposed to

**2.8 Plasmid Profile of Multiple Antibiotic Resistance Isolates**

**2.8.1 Plasmid DNA Extraction**

The isolates with MAR index 0.6 were subjected to plasmid DNA isolation using Inqaba Biotec West Africa Ltd provided Bacterial plasmid MiniPrep$^{TM}$ Kits (Code No. ZR D6005, made in USA by Zymo Research) Following the manufacturer specification. Each isolate was inoculated into 10ml Luria-Bertani (LB) broth incorporated with appropriate selection antibiotic and incubated for 16hours at 37°C while shaking at 200-250rpm. The bacterial culture was harvested by centrifugation at 8000rpm in a microcentrifuge (Model 5417R) for two minutes at room temperature. The supernatant was decanted and all remaining medium removed. The pelleted cells were resuspended in 250µl of resuspension solution and transferred to microcentrifuge tube. Exactly 250 µl of lysis solution was added and mixed thoroughly by inverting the tube 4-6 times until solution was viscous and slightly clear. This were followed by adding 350 µl of neutralization solution and mixed by inverting the tube. Centrifugation was carried out at 10000rpm for five minutes to pellet cell debris and chromosomal DNA. The supernatant was transferred to GeneJET spin column by decanting.

Centrifugation was carried out for one minute and the flow-through was discarded. Wash solution of 500 µl was added to the column and centrifuged for 30 to 60 seconds, flow-through was discarded and column placed back into collection tube. The wash procedure was repeated to avoid residual ethanol in plasmid preps. The Gene JET spin column was transferred into fresh 1.5 ml micro centrifuge tube and 50µl of elution buffer was added to the center of the column to elute plasmid DNA. This was incubated for 2 minutes at room temperature and centrifuged for 2 minutes. The purified plasmid DNA was stored at -20°C for further studies.

**2.8.2 Detection of number and sizes of Plasmid DNA (Agarose Gel Electrophoresis)**

One per cent (1.0%) agarose gel (Schwarz/Mann Biotech) was used to resolve DNA fragment. This were prepared by combining 1g agarose in ten times concentration of tris-borate ethylene diamine tetraacetate (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave ( Townson/Mercer Ltd. Croydon, England) for 2 minutes until the agarose is dissolved (Moore *et al*., 2002).
Exactly 0.5 µl of Ethidium bromide were added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs were inserted. The gel was allowed to gel for 30 minutes. The casting combs were carefully removed after the gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 0.5 µl of gel tracking dye (bromophenol blue) was added to 20 µl of each sample with gentle mixing. The samples were loaded onto the wells of the gel at a concentration of 20 µl, the mini horizontal electrophoresis gel setup will be covered and electrodes connected. Electrophoresis was carried out at 100-200mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and viewed under UV-transilluminator (302nm) (Vilber Lourmat, Germany). The band pattern of DNA fragments were photographed with a Polaroid camera and documented using electrophoresis gel documentation system.

## III. RESULT

**Isolation and Identification of *Escherichia coli***

The cultural, morphological and biochemical characteristics of *Escherichia coli* isolated from faecal matter obtained from Abattoir in Keffi metropolis, Nigeria as shown in Table 1.

**Occurrence of *Escherichia coli***

Out of fifty (50) samples of faecal matter from Abattoir obtained, 100% of *Escherichia coli* were isolated from the faecal samples as given in table 2.

**Antibiotic Resistance**

The *Escherichia coli* isolated from feacal samples from Abattoir in Keffi metropolis were highly resistance to nalidixic acid, cefotaxine, ceftazidine, streptomycine and ceftriaxone, sulphamethoxazole/trimethoprim and with percentage resistance

of 98.0%, 96.0%, 82.0%, 65.0% 40%, and 40% but less resistance to ciprofloxacin and, amoxicillin/clavulanic acid, imipenem and gentamicin with percentage resistance of 36.0%, 12%, 14.0% and 8% as shown in Table 3.

**Antibiotic Resistance Pattern**

The antibiotic Resistance pattern of *Escherichia coli* isolated from faecal matter of Abattoir in Keffi metropolis as shown in table 4. The *E. coli* isolates were distributed into different antibiotic resistant pattern and the most common pattern was NA-S-CRO-CTX-CAZ-SXT and CRO-CTX-SXT-CN-AMC-CIP-IPM-S-CAZ with a resistance of 12% as shown in Table 4.

**Multiple Antibiotic Resistance (MAR) index**

The MAR index of *Escherichia coli* isolated from faecal matter from Keffi Abattoir as shown in Table 5. all the *Escherichia coli* isolates were MAR isolates with MAR index of ≥ 0.2 and the most common MAR index was 0.6 and percentage of it occurrence was 8(16.0%) as shown in table 5.

**Plasmid profile of Antibiotic Resistance of *Escherichia coli***

**4.1.6 Plasmid profile of Antibiotic Resistance of *Escherichia coli***

The agarose gel electrophoresis resolution of plasmid DNA from multiple antibiotics resistant *E. coli* isolates with MAR index of 0.6 showed that Lanes p1, p2, p3, p4, p5, p6, p7 and p8 of test *E.coli* isolates displayed one plasmid each with the same molecular weights of 1200bp as shown (Plate 1) .

**Table 1:Cultural Morphological and Biochemical characteristics of *Escherichia coli* Isolated from Faecal Matter in Abattoir Keffi Metropolis, Nigeria**

| Cultural characteristics | Mophological characteristics GramReaction morphology | Biochemical characteristics Ind Mr Vp CT ONPG Orn Ur Nt $H_2S$ Mal | Inference |
|---|---|---|---|
| Pinkish colonies on MCA and greenish metallic sheen colonies on EMB agar | - rod shape | + + - - + + - + - - | *E. coli* |

- = negative; + = Positive; ind = Indole; mr = methyl red; V= Voges Proskauer; CT = Citrate; orn = ornithine; onpg = ur = urease; Nt = nitrate; $H_2S$ = Hydrogen sulphate; mal = malonate

**Table 2: Occurrence of *Escherichia coli* from faecal matter from Abattoir in Keffi metropolis, Nigeria**

| Samples | No. of Samples | N0. ( % ) *E. coli* |
|---|---|---|
| Faecal matter | 50 | 50 (100.0) |

**Table 3: Antibiotic Resistance of *Echeriachia coli* isolated from faecal matter from Abattoir from Keffi metropolis, Nigeria.**

| Antibiotic | Disc content (μg) | No. (%) Resistant ( n=50) |
|---|---|---|
| Amoxycillin | 30 | 6 (12.0) |
| Ceftazidine | 30 | 41 (82.0) |
| Gentamicin | 10 | 4 (8.0) |
| Ceftriaxone | 30 | 20 (40.0) |
| Cefotaxacin | 10 | 48 (96.0) |
| Streptomycin | 30 | 30(60.0) |
| Imipeneme | 30 | 7 (14.0) |
| Sulphamethoxazole | 25 | 20 (40.0) |
| Nalidixic Acid | 30 | 49 (98.0) |
| Ciprofloxacin | 10 | 18 (36.0) |

**Table 4: Antibiotic Resistance pattern of *Escherichia coli* from feacal matter from Abattoir in Keffi metropolis, Nigeria**

| Antibiotic Resistance pattern | Frequency (%) Isolates, (n = 50) |
|---|---|
| NA | 2 ( 4.0 ) |
| S, CAZ | 3 ( 6.0 ) |
| NA, CTX | 3( 6.0 ) |
| NA, CTX, CAZ | 1 ( 2.0 ) |
| NA, S, CAZ | 1 ( 2.0 ) |
| NA, AMC, S | 1 ( 2.0 ) |
| NA, S, CRO | 1 ( 2.0 ) |
| NA, SXT, CTX | 1 ( 2.0 ) |
| NA,CIP,CAZ, CTX | 1 ( 2.0 ) |
| NA,S,SXT,CAZ | 1 ( 2.0 ) |
| NA,CIP,SXT,CTX | 1 ( 2.0 ) |
| NA,SXT,CAZ,CTX | 1 ( 2.0 ) |
| NA,CTX,CRO,S | 1 ( 2.0 ) |
| NA,SXT,CTX,S | 1 ( 2.0 ) |
| NA,SXT,S,CN,CAZ | 1 ( 2.0 ) |
| CIP,NA,SXT,CAZ,CTX | 1 ( 2.0 ) |
| NA,SXT,CAZ,S,CTX | 1 ( 2.0 ) |
| NA,CRO,CAZ,S,CTX | 1 ( 2.0 ) |
| NA,S,CRO,CAZ,SXT | 2 ( 4.0 ) |
| NA,S,SXT,CAZ,IPM | 1 ( 2.0 ) |
| NA,CIP,SXT,S,IPM,CTX | 1( 2.0 ) |
| NA,S,CRO,CTX,CAZ,SXT | 6 ( 12.0 ) |
| NA,CIP,S,CRO,CAZ,SXT | 1 ( 2.0 ) |
| NA,S,CRO,CTX,CAZ,CN,SXT | 1 ( 2.0 ) |
| CRO,CTX,SXT,AMC,CIP,CAZ,NA | 3 ( 6.0 ) |
| CRO,CTX.SXT.AMC,CIP,CAZ,IPM | 1 (2.0) |
| NA,CRO,CTX.SXT.AMC,CIP,CAZ,IPM | 5 (10.0) |
| CRO,CTX,SXT,CN,AMC,CIP,IPM,S,CAZ | 6 ( 12.0 ) |

**Table 5: Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* isolated from faecal matter from abattoir in Keffi metropolis, Nigeria**

| No.0f Antibiotic Resistance to (a) | No. of Antibiotic tested (b) | MAR Index (a/b) | Frequency (%) MAR isolates (n= 50) |
|---|---|---|---|
| 9 | 10 | 0.9 | 6(12.0) |
| 8 | 10 | 0.8 | 5(10.0) |
| 7 | 10 | | |
| 6 | 10 | 0.7 | 5(10.0) |
| 5 | 10 | | |
| 4 | 10 | 0.6 | 8(16.0) |
| 3 | 10 | | |
| 2 | 10 | 0.5 | 7(14.0) |
| 1 | 10 | | |
| | | 0.4 | 6(12.0) |
| | | 0.3 | 5(10.0) |
| | | 0.2 | 6(12.0) |
| | | 0.1 | 2(4.0) |

MAR = Multiple Antibiotic Resistance

**Table 6: Numbers and Size of plasmid of MAR of *Escherichia coli* isolated from faecal matter from Abattoir in Keffi metropolis, Nigeria**

| ISOLATES | Number of Plasmids | Estimated Molecular sizes in base pairs (bp) |
|---|---|---|
| **P1** | 1 | 1200 |
| **P2** | 1 | 1200 |
| **P3** | 1 | 1200 |
| **P4** | 1 | 1200 |
| **P5** | 1 | 1200 |
| **P6** | 1 | 1200 |
| **P7** | 1 | 1200 |
| **P8** | 1 | 1200 |

P1 to p8 represent the plasmid isolates, M represent a molecular larder.

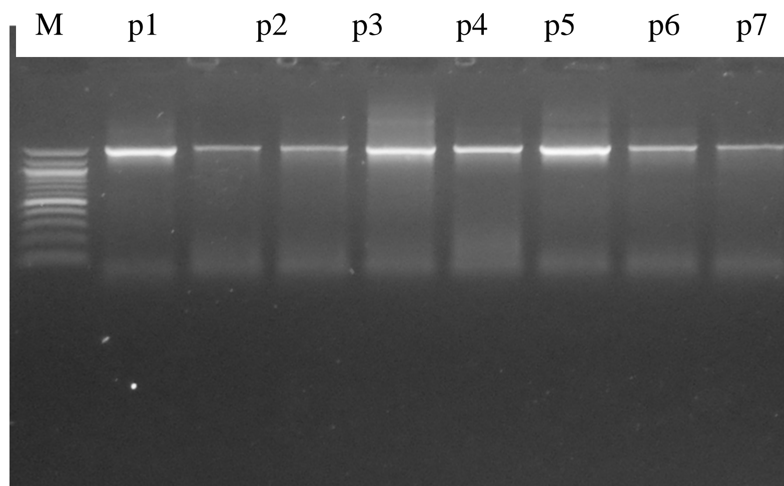


**Plate 1: Agarose gel electrophoresis of the plasmid of the bacterial isolates in lane p1 to p8 while Lane M represents a 1500 molecular ladder**

## IV. DISCUSSION OF FINDINGS

Antimicrobial use in animal production has been shown to lead to the emergence of resistant *Escherichia coli* throughout the food chain (Blanco *et al.,* 2011). Abattoirs are known all to pollute the environment either directly or indirectly from their various processes (Iroha *et al*., 2016). This study focuses on the Antibiotic Resistance and plasmid profile of *E. coli* isolated from faecal matter from Abattoir in Keffi metropolis, Nigeria. The prevalence of *E. coli* in faecal samples (100%) in this study was higher than the previous records of 60% and (Nazir, 2007) and (Rahman *et al.,*2008). The occurrence of *E. coli* from faecal matter obtained in not different from the study earlier reported by (Alhaj *et al*., 2007). The occurrences of *E. coli* from faecal matter observed in this study justify the fact that the cattle are reservoir of this organism. The occurrence of *E. coli* from these sources may have public health implication, since *E. coli* have been reported by many researchers as the most common agent of food borne diseases (Legesse *et al*., 2015). The result of our findings of antibiotic resistance shows that the isolates were highly resistant to Nalidixic Acid, ceftriaxone, ceftazidime and streptomycin and these finding is with agreement with the study earlier described by (Alhaj *et al*.,2007; Babu *et al.,* 2009). Multiple resistances were reported to be more common than resistance to single antibiotics. It was also reported that a high percentage of *E. coli* isolated from faecal samples were resistant to one or more antibiotics and MDR isolates were more common with occurrence of 84.0%. This however is in agreement with studies earlier described by (Nsofor & Iroegbu, 2012, Adzitey *et al.,* 2012). The high resistance of *E. coli* isolates to antibiotics mentioned may be due to use of antibiotics as growth promoting substances in animal husbandry. The low susceptibility of *E. coli* to ceftriaxone, ceftazidime and ciprofloxacin may be due to misuse or abuse of the antibiotics (Ngwai *et al*., 2011; Okeke *et al.,* 2007). The percentage resistance of *E. coli* isolates to ciprofloxacin and third generation cephalosporin which are ceftriaxone 40%, ceftazidime 82% and cefotaxime 96% observed in this study were higher than 34% and 8.6 % reported by (Monique *et al* 2016). The higher level of resistance to third generation cephalosporins observed in this study may be due to the production Extended-spectrum beta-lactamases enzymes, although from the study the production of ESBL by *E.coli* Isolates resistance to third generation cephalosporin were not valuated in this study.

The number and sizes of plasmid detected in multiple antibiotic resistance *E.coli* isolates observed in this study was different from the study earlier described by (Nsofor & Iroegbu, 2013) with size range of 1000bp to 120000bp and similar to study earlier described by (Adzitey *et al.,* 2012) in term of the plasmid size of 1200bp. The detection of plasmid DNA in the MAR isolates observed in this study is an indication that the genes may be encoded in the plasmid although the conjugation studies on the transfer of plasmid mediated resistant genes were not evaluated in this study. The low resistance of *E. coli* isolates to gentamicin 4%, amoxicillin/clavulanic acid 6% and imipenem 7% observed in this study was expected and this is similar to the study earlier described by Adzitey *et al.* (2012d) and Adzitey *et al.* (2012a). The low resistant of imipenem was with agreement to the study earlier described by Ajay *et al.* (2013) but was in contrast to Uma *et al.* (2009) who reported high resistance to imipenem. The percentage resistance of the isolates to gentamicin, amoxicillin/clavulanic acid imipenem observed in this study was lower than the 12.5%, 58.8% and 20% of study earlier described by Nsofor and Iroegbu, (2013) and Ajay *et al.* (2013). The low susceptibility of *E. coli* to Ceftazidime observed in this study was in agreement with the study earlier reported by Ngwai *et al* (2011). The low percentage resistance of the isolates of the antibiotic reaction is an indication that such antibiotic may not have been abused or misused in the study location.

## V. CONCLUSION

The *E. coli* isolates were less resistance to gentamicin, imipenem and amoxycilin/clavulanic acid and in view of this low resistance, such Antibiotic may be useful for treatment of infection cause by *E.coli* . In addition the MAR *E.coli* isolates carries the plasmid DNA and may be responsible for antibiotic resistance. Base on the findings of the study, the following are recommended:

i. There should be mass education and public awareness programs on abuse and misused of antibiotics in Animals farms and slaughter house.The education and communication on the issue of antibiotic used in veterinary medicine should be improved.

ii. There is need for constant antimicrobial susceptibility surveillance on cattle in slaughter house in Keffi, Nasarawa State.

iii. Excess use or abuse of antibiotics should be reduced or stopped by judicious application of antibiotics for the safety of humans and farm animals. Further studies on molecular diversity of the resistance isolates should be carried out.

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