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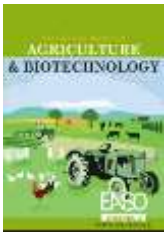

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Original Article

Antimicrobial Activity of Oxytetracycline on *Escherichia Coli* Isolated from Chicken in Sokoto, Nigeria

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Chicken, Escherichia coli, Minimum Inhibitory Concentration, Oxytetracycline, Sokoto.

ABSTRACT

This study explored the prevalence and pattern of antimicrobial activity of Oxytetracycline on Escherichia coli (E. coli) isolated from chicken presented with cases of diarrhoea at the Avian Clinic of the Veterinary Teaching Hospital, Usmanu Danfodiyo University, Sokoto. Cloacal swab samples were taken from 50 chickens of various types and age groups with suspected Colibacillosis. Bacterial culture and identification was carried out, 72% of the isolates were presumed to be E. coli, with 40% confirmed after biochemical characterization. Distribution analysis indicated highest prevalence of the organism in layers (8 isolates), followed by broilers (7), local chicken (2), cockerels (2), and lastly noilers (1). No statistical significant differences were found among types of chicken using Chi square with $P > 0.05\%$. Regarding age, 40% of the isolates were from young chicken, and 60% were from adult ones, with no statistical significant difference observed among them ($P > 0.05\%$). Additional investigations, including polymerase chain reaction (PCR) analysis, were conducted to further confirm the isolates. Oxytetracycline susceptibility test to elucidate antibiotic resistance in these E. coli isolates revealed 30% sensitivity and a concerning 70% resistance, indicating a challenge in managing E. coli infections in chicken in Sokoto using oxytetracycline. E. coli isolated from commercial (exotic) chicken were found to be more resistant to oxytetracycline than the ones isolated from local chicken (77.8% and 0% respectively). E. coli isolates from adult chicken were found to be more resistant to oxytetracycline than the ones isolated from young chicken (75% and 62.5% respectively). In conclusion; this study identifies the substantial presence of oxytetracycline-resistant E. coli strains in chicken in Sokoto. Vigilant monitoring and judicious antibiotic use are crucial to curb further antibiotic resistance spread in poultry through creation of awareness among farmers and veterinary drug vendors about dangers associated with antibiotic resistance. Further in vivo studies should be undertaken to correlate the in vitro susceptibility results to help validate the findings and guide future treatment protocols.

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INTRODUCTION

In many developing regions, the practice of smallholder poultry rearing is widespread, occurring in both rural and some urban areas. The demand for poultry is on the rise due to factors such as population growth, escalating urbanization, and increased incomes (Dawe, 2014). The importance of poultry to Nigerian economy cannot be over emphasized as it has become popular for the small-holders that have contributed to the economy of the country. In Nigeria, most poultry farmers focus on rearing chicken and turkeys, with chicken being the most preferred. About 85 million (42%) of Nigeria's population, 4 in every 10 Nigerian, are into poultry production, primarily small-scale to medium-scale poultry farming. Broilers make up 70% of the chicken population in Nigeria, while layers account for 30% (Adeite, 2021).

The poultry sector is challenged with some common diseases that are responsible for decreased production which in turn lead to economic losses. Colibacillosis is one of the commonest and principal causes of morbidity and mortality in young chicks **(Saberfar *et al.*,** 2008**)**. The condition has been associated with heavy economic losses in the poultry industry by its association with various disease conditions, either as a primary or secondary pathogen **(Saberfar *et al.*, 2008)**. Colibacillosis is most commonly seen following upper respiratory tract infection (such as Infectious Bronchitis or Mycoplasmosis). It is also associated with immunosuppressive diseases such as Infectious Bursal Disease (Gumboro Disease) in chicken, *Haemorrhagic enteritis* in turkeys and in young birds that are immunologically immature (Nolan *et al*., 2015). Colibacillosis is caused by the bacterium *Escherichia coli*, which has been reported worldwide in chicken and turkeys (Kabir, 2010). The disease in poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by pericarditis, airsacculitis and perihepatitis (**Saberfar 2005**). The disease is usually seen in young chicks up to three weeks of age. Enterotoxigenic *E. coli* strains have been seldom isolated in chicken suffering from diarrhoea and occasionally in clinically healthy chicken and turkeys (Kabir, 2010). Infection through the oral or inhalation routes and via shell membranes, water and fomites has been reported, with an incubation period of 3-5 days. Morbidity varies but mortality ranges from 5-20%. Avain pathogenic *E. coil* (APEC) has been incriminated in a lot of cases of *E. coli* infection in both broilers and layers. It causes embryo mortality and omphalitis in chicks. Lesions observed are mainly polyserositis with deposition of fibrin in the air sacs, pericardium and liver (Ardrey ***et al.*,** 1968). Polymerase chain reaction (PCR)

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should be used as a routine diagnostic technique for rapid detection of *E. coli* in clinical samples (Abdelrahman, 2008).

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome (Bolouri *et al.*, 2016). All groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) are in danger of losing their efficacy because of the increase in microbial resistance (Mayers *et al*., 2009). Oxytetracycline is one of the drugs that are commonly used and misused in veterinary practice, especially poultry farmers who use it as feed additive. Oxytetracycline is a highly active broad-spectrum antibiotic that works by inhibiting protein synthesis. Other similar drugs include Chlortetracycline, Tetracycline, and Doxycycline (Casewell *et al.*, 2013). The drug has activity against gram-positive and gram-negative bacteria, including some anaerobes. It is also active against Chlamydia, Mycoplasmas, and some Protozoa, and several Rickettsiae (McCormick and Johnson 2013). Specific bacteria within the tetracycline's activity range include *Escherichia coli, Klebsiella species, Pasteurella species, Salmonella species, Staphylococcus species, and Streptococcus species* (McCormick and Johnson 2013). Oxytetracycline can be given in drinking water and feed, through subcutaneous or intramuscular route. With intramuscular administration, there is a risk of muscle irritation or necrosis. After intramuscular administration of oxytetracycline, peak levels may occur in 30 minutes to several hours, depending on the volume and site of injection (Casewell *et al.*, 2013). There are several methods of antimicrobial susceptibility testing with broth micro- or macro-dilution as one and the most basic method (Bolouri *et al.*, 2016).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that prevents visible growth of a microorganism after overnight incubation with media. To determine MIC of an antimicrobial agent, the organism, the antimicrobial agent and media are required. MIC values provide quantitative measures of antimicrobial potency of a drug, the lower the MIC, the higher the potency of the antimicrobial (Kowalska-Krochmal and Dudek-Wicher, 2021).

The study aimed at determining the in-vitro antimicrobial activity of oxytetracycline on *E. coli* isolated from chicken presented at Veterinary Teaching Hospital (VTH) Usmanu Danfodiyo University, Sokoto (UDUS).

MATERIALS AND METHOD

Study design

The study was designed to determine the antimicrobial activity of oxytetracycline on *E. coli* isolated from chicken presented at Veterinary Teaching Hospital Usmanu Danfodiyo University, Sokoto. The study focused primarily on the prevalence of susceptibility and minimum inhibitory concentration of oxytetracycline on the *E. coli* isolates. All samples were properly labeled to avoid duplication of isolates of *E. coli.*

Sampling frame

The research was carried out in Sokoto State, Nigeria. Fecal samples that were watery or bloody were taken from 50 indigenous and commercial chickens presented with diarrhoea characteristic of Colibacillosis at Avian Clinic of Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto. Out of the 50 samples collected within the period of 3 months, 29 (58%) were adult and 21 (42%) were young. 40 (80%) were exotic (5 cockerels, 15 layers, 20 broilers), 5 (10%) were cross (noilers) and 5 (10%) were local chickens mostly backyard poultry. The numbers of different variables were as a result of obtained cases.

Samples collection and Handling

The chickens were properly restrained and sterile swab sticks were used to swab the cloaca. The swab stick containers were labelled using codes to represent type of chicken and age. The labelled

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samples were then transported immediately to the Veterinary Microbiology Laboratory UDUS and preserved in refrigerator at 4°C before analysis.

Sample Processing

A volume of approximately 1 ml of inoculums from a pure culture was aseptically transferred into 9 mls of buffered peptone water and incubated at 37°C for 24 hours. Subsequently, a loopful of each sample was streaked onto prepared MacConkey agar plates and then incubated at 37°C for an additional 24 hours. The plates were meticulously examined for the emergence of pinkish colonies, indicative of Lactose Fermenters. The colonies exhibiting lactose fermentation were subjected to Gram staining, and gram-negative rods observed under a light microscope were carefully chosen for sub-culturing.

Screening of suspected isolates on eosin methylene blue (EMB) plate

Lactose fermenting colonies on MacConkey plate were streaked onto EMB agar plate for presumptive confirmation as *E. coli*. Purplish colonies associated with green metallic sheen were picked and streaked on nutrient agar slant, incubated at 37^0C for 24 hours and stored at 4^0C pending further biochemical characterisation of *E.coli*.

Biochemical Characterization

The isolates were further subjected to biochemical identification using triple sugar iron, indole, methyl red, voges proskauer, citrate and urease. This was by indol production, fermentation of glucose with gas production, presence of ß-galactosidase, absence of hydrogen sulphite production and urease and the inability to use citrate as a carbon source. The Interpretation of results was based on change in colouration observed during the various steps of the test indicating positive or negative.

DNA Extraction

The DNA of the Phenotypically identified *E.coli* isolates were extracted using Qiagen® DNA extraction kit, following manufacturers instruction.

Polymerase chain reaction (PCR) Amplification of UidA gene of *Escherichia coli*

The presence of resistance UidA gene was determined by conventional PCR after *E. coli* isolation. A total of 25 micro-liter reaction was prepared in a 0.2 ml PCR tube containing the following components; 12.5uL of Super-mix (Transgene® China), 0.5uL of UidA forward and reverse primers to amplify a 166 base pair DNA fragment (Table 1), 8uL of nuclease free water and 4uL of DNA template. The tubes were centrifuged briefly and transferred into a Biorad® thermal cycler Programmed with the following cycling condition: Initial denaturation of 94ºC for 5 minutes followed by 35 cycles of denaturation of 94ºC, annealing at 63ºC for 30 seconds, extension at 72ºC for 1 minutes followed by final extension at 72ºC for 5 minutes. The amplified products were resolved in a 1% agarose gel pre-stained with ethidium bromide in a 1X TAE buffer and the electrophoresis was carried out at 100 volts for 45 minutes. The gel was viewed in a gel documentation device using UV trans-illuminator. Table 1 shows the primers used in carrying out the PCR.

Table 1: Primers used for PCR

Primer	Primer sequences (5′ to 3′)	Position on gene
uidA Up	tatggaatttcgccgatttt	1939- 1958
uidA Down	tgtttgcctccctgctgcgg	2084- 2104

Broth microdilution assay

Oxytetracycline powder (909 mg) was dissolved in 10ml of distilled water. The working concentration was prepared using $C_1V_1=C_2V_2$ where C and V mean concentration and volume respectively and 1 and 2 mean initial and final respectively. A double-strength Muller Hinton broth was prepared following the manufacturer's instructions and

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adjusted to a turbidity matching the 0.5 MacFarland standard. Using a micro pipette, 50µl of this suspension was transferred into all 96 wells of a microplate. Subsequently, 50µl of the oxytetracycline solution was dispensed into well 1A, mixed with Muller Hinton broth, and 50µl from well 1A was aspirated and transferred to well 2A. This serial dilution process continued up to well 11A, with well 12 serving as a control. The same procedure was applied to rows B, C, D, E, F, G, and H on the microplate. To each well, 50µl of the suspected *E. coli* isolates was added, and the microplates were covered and incubated at 37°C for 24 hours. Iodo Nitro Tetrazolium (INT) served as growth indicator that turn yellowish to pinkish or reddish on the microplate. The minimum inhibitory concentrations of oxytetracycline against the *E.coli* isolates were determined.

DATA ANALYSIS

The data was imputed in Microsoft excel, and Chi square was used to determine the association between variables, with probability value of less than 0.05 considered statistically significant.

RESULTS

A total of 50 cloacal swab samples collected from the chickens diagnosed with Avian Colibacillosis and subjected to bacteriological culture and identification, revealed 20 (40%) to be identified as *E.coli* using biochemical means while 30 (60%) were not *E.coli.*

Overall Isolation Rate of *Escherichia coli* from Cloacal swabs of Chickens

Out of the 50 cloacal swab samples subjected to bacterial culture and identification, 37 (72%) were lactose fermenters while 20 (40%) were identified as *E.coli* using biochemical means (table 2).

Table 2: Isolation Rate of *Escherichia coli* from feces of Chickens

Number Tested	Positive (%)	Negative (%)
50	20 (40)	30 (60)

Isolation Rate of *Escherichia coli* Based on Type of Chicken

Exotic, cross breed (noilers) and local chicken were sampled in the study. For cockerels, layers, broilers and noilers 40.0%, 57.1%, 35.0% and 20.0% tested positive respectively, while 33.3% local chicken tested positive (Table 3).

Table 3: Isolation Rate of *E. coli* Based on Type of Chicken

Type of Chicken	Number Tested	Positive (%)	Negative (%)
Cockerels	5	2 (40.0)	3 (60)
Layers	14	8 (57.1)	6 (42.9)
Broilers	20	7 (35.0)	13 (65)
Noilers	5	1 (20.0)	4 (80)
Local Chicken	6	2 (33.3)	4 (67.7)
Total	50	20 (40.)	30 (60)

Statistically, there was no significant difference between the types with Chi-value 2.9656, CI (Confidence interval) 95% and P value being 0.5634.

Age specific isolation rate

Out of the 50 samples, young chicken had 8 (21.6%) positives and adult chicken had 12 (32.4%) positives (table 4).

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Table 3: Isolation Rate of *E. coli* Based on Age Distribution

Age	Number tested	Positive (%)	Negative (%)
Young	21	8 (38.1)	13 (61.9)
Adult	29	12 (41.4)	17 (5.6)
Total	50	20	30

There was no statistically significant difference with Chi-value 0.9421, CI (confidence interval) 95% and P value being 1.0000.

After PCR was conducted where 166bp was amplified using UidA primers. Figure1 shows the amplification products for the isolated *E. coli*.

PCR Gel Imaging

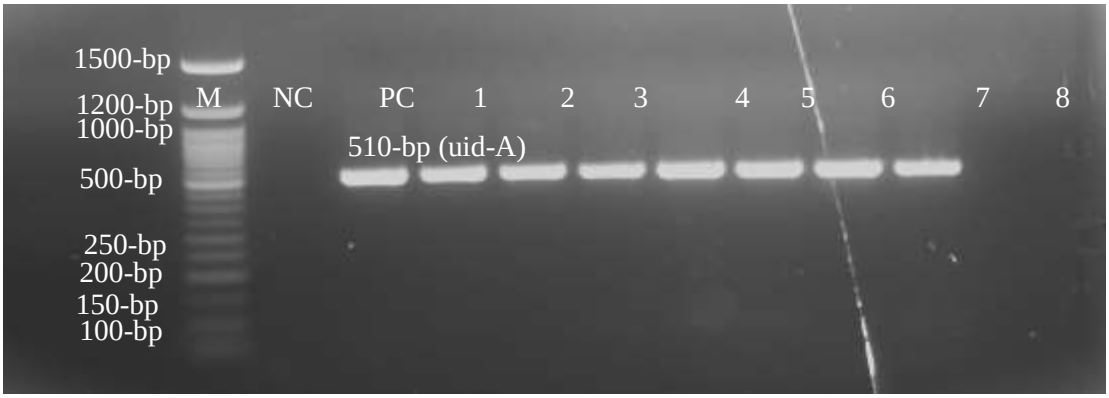

Figure 1: Representative image of the PCR products observed on 1% agarose gel showing amplified *E.coli* uid-A gene corresponding to the positive control and targeted position on DNA ladder.

The Minimum Inhibitory Concentration of Oxytetracycline on *E. coli* Isolates

Out of the 20 *E. coli* isolates, 6 (30%) were susceptible to oxytetracycline, 14(70%) were resistant (figure 2). Resistant = ≥ 0.016µg/µl and susceptible is < 0.016µg/µl from the reference given by the Clinical and Laboratory Standard Institute (CSLI) (table 4).

Table 4: Minimum Inhibitory Concentration of Oxytetracycline on *E. coli* Isolates

Isolate	M.I.C (µg/µl)	Status
L/A/01	0.625	Resistant
L/A/03	1.25	Resistant
L/A/04	2.5	Resistant
L/A/07	0.625	Resistant
L/A/08	0.0049	Susceptible
L/A/09	0.625	Resistant
L/A/12	1.25	Resistant
L/A/14	2.5	Resistant
C/A/02	1.25	Resistant
C/A/04	0.0049	Susceptible
B/Y/15	0.313	Resistant
B/Y/19	0.078	Resistant
B/Y/02	0.0049	Susceptible
B/Y/03	1.25	Resistant
B/Y/06	0.313	Resistant
B/Y/07	0.0049	Susceptible

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B/Y/14	0.313	Resistant
N/A/04	0.625	Resistant
LC/A/01	0.0049	Susceptible
LC/Y/05	0.0049	Susceptible

Key: L= Layer, C= Cockerel, B= Broiler, N= Noiler and LC= Local Chicken, Y= Young, A= Adult.

Oxytetetracycline Antimicrobial Activity Pattern on *Escherichia coli* Isolated from different categories of Chickens

Out of the 20 *E. coli* isolates, 37.5% from young chicken were susceptible and 62.5% were resistant while 25% from adult chicken were susceptible and 75% were resistant to oxytetracycline (Table 5). On types of chicken, 100% from local chicken were susceptible and 0% was resistant while 22.2% from commercial chickens were susceptible and 77.8% were resistant (Table 6).

Figure 2: Antimicrobial susceptibility test of *E.coli* to Oxytetracycline in tested chicken.

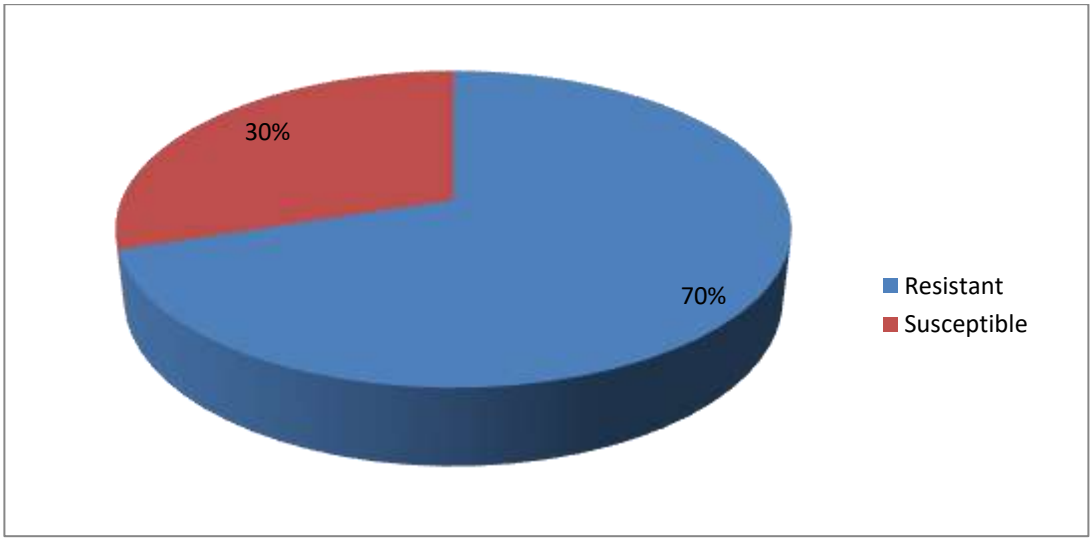

Table 5: *Escherichia coli* Sensitivity to Oxytetetracycline Pattern in Different Age Groups of Chicken

Age	Number obtained	Susceptible (%)	Resistant (%)
Young	8	3 (37.5)	5 (62.5)
Adult	12	3 (25.0)	9 (75.0)
Total	20	6 (30.0)	14 (70.0)

Table 6: *Escherichia coli* Sensitivity to Oxytetetracycline Pattern in Different Types of Chicken.

Age	Number obtained	Susceptible (%)	Resistant (%)
Commercial	18	4 (22.2)	14 (77.8)
Local	2	2 (100.0)	0 (0.0%)
Total	20	6	14

Discussion

From this research, a total of 37 (72%) out of 50 cloacal swab samples taken from chicken suspected with Colibacillosis in VTH UDUS expressed lactose fermentation, and 20 (40%) of the isolates were biochemically confirmed as *E. coli*. This is the first known study where invitro antimicrobial

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activity of Oxytetracycline was tested on isolated *E. coli* from chicken in Sokoto. The prevalence obtained can be associated with poor managemental practices especially poor hygiene in poultry houses coupled with inadequate consultations of veterinarians by poultry farmers.

In this study, adult birds mostly layers were found to have the highest positive result for *E. coli* 57.1%, followed by commercial cockerels with 40%, broilers 35%, local chickens 33.3% and noilers having the least (20%) though with no significant statistical difference using Chi square. Nolan *et al.* (2013) reported that Colibacillosis is one of the most common infectious bacterial diseases of the layer industry. Among the predisposing factors of the disease is stress of production in young developing birds. Inhalation of contaminated dust is the most likely source of *E. coli* infection (Colibacillosis) for poultry. Charlton (2006) reported that adverse reactions from routine vaccinations can also cause damage to the respiratory tract and this has the potential to allow pathogenic bacteria to enter the blood stream which can lead to septicaemia. The disease causes elevated morbidity and mortality leading to economic losses on a farm especially around the peak of egg production and throughout the late lay period.

Antibiotic usage is possibly the most important factor that promotes the emergence, selection, and dissemination of antibiotic-resistant microorganisms in veterinary medicine. In poultry flocks, inappropriate antibiotic therapy and using antibiotics as growth promoters may result in high antibiotic selection pressure. Oluwasile *et al*., (2014) and Agyare *et al* (2019) reported that almost all known antibiotics are increasingly losing their activity against pathogenic microorganisms. The levels of multi-drug resistant bacteria have also increased. The MIC and checkerboard are employed tests for determining the sensitivity of *E. coli* isolates to different antimicrobial agents

In this study, there was high resistance (70%) of *E. coli* to oxytetracycline, 75% resistance among adults, 62.5% among young, 77.8% among commercial and 0% among local chickens. Aworh *et al.* (2019) reported high prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria. Also, Aworh *et al.* (2020) reported high prevalence (37.8%) of Extended-spectrum ß-lactamase-producing *Escherichia coli* in chickens in Abuja Nigeria where tetracycline, sulphonamides and aminoglycosides class of antimicrobials accounted for the majority of the resistance determinant. Adebowale *et al.* (2022) reported 80% and 56.3% *E. coli* prevalence and multidrug resistant *E. coli* respectively in chickens from live bird markets in Ogun state, Nigeria. Ojo *et al.*, (2012) reported 76.9% *E coli* resistance to tetracycline in free-range chickens in Abeakuta Nigeria.

The reported resistance of *E. coli* to oxytetracycline in other similar studies in other parts of the world is in line with the findings of this work. Aggad *et al.*, (2010) reported 87% oxytetracycline resistance by *E. coli* from broilers with clinical signs and lesions of Colibacillosis in west area of Algeria. Rahimi (2013) reported *E. coli* strains in poultry in Iran with distinctively high resistance rates of 85.1% to tetracycline. Also, Aberkane *et al*. (2023) reported 100% resistance of *E. coli* isolated from slaughtered chickens in Batna city of Algeria to oxytetracycline by disc diffusion method. The study by Das *et al*. (2020) on commensal *Escherichia coli* strains in broiler chickens and farm environments in Bangladesh revealed that all *E. coli* isolates, regardless of the source of origin, were resistant to oxytetracycline. Study by Fairchild *et al.* (2005) where effects of tetracycline administration on commensal bacteria from commercial poultry was investigated, revealed that *Enterococcus* spp. and *E. coli* were resistant to tetracyclines with 32.2% harboring *tet*(A) and 30.5% containing *tet*(B) resistance genes.

Pereira *et al.* (2024) reported percentage of *E. coli* isolates with MDR based on the DDT to be

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significantly higher in broiler chickens and layer farms than in local chickens. This is similar to the result of this study where resistance of *E. coli* to oxytetetracycline was seen in layers and broilers, whereas local chickens were susceptible. The higher resistance in layers obtained in this study could be attributed to the routine use of antimicrobials in commercial poultry rearing which is a common practice and eventually leads to day-old chicks becoming source of antimicrobial resistant bacteria for chicken farms as reported by Moreno *et al.* (2019) and Okorafor *et al.* (2019).

These obtained high resistance could be attributed to indiscriminate use of antibiotics by poultry farmers especially oxytetracycline as prophylactic or therapeutic agent and sometimes as feed additive. This study shows the use of Oxytetracycline at therapeutic doses in poultry production can lead to the emergence and persistence of resistant *E. coli* strains, which can be a risk to both human and animal health as reported by Pokrant *et al.,* (2023) where effect of Oxytetracycline on selection of resistant *Escherichia coli* was assessed in broiler chickens.

Conclusion

From the result of this study, *Eschericia coli* was prevalent among all types and age groups of chickens presented at the VTH UDUS with clinical signs of Colibacillosis which were confirmed using PCR. The prevalence rate was low but there was high resistance of the bacteria to Oxytetracycline after determination of minimum inhibitory concentration of the antibiotic on the confirmed isolates.

Recommendations

Based on the findings of this research work showing high resistance of *Eschericia coli* to Oxytetracycline, it is recommended that awareness should continue to be created among farmers and veterinary drug vendors about dangers associated with antibiotic resistance. Also there should be promotion of rational use of antimicrobials, as it is not sufficient to quantitatively reduce antibiotic consumption but to qualitatively improve their usage**.** Similar research should be carried out in the study area with the same antimicrobial on other bacterial organisms or different antimicrobial against the same or different common pathogenic bacteria agents of poultry. Further in vivo studies should be undertaken to correlate the in vitro susceptibility results with in vivo studies. This can provide valuable insights into how antimicrobials behave within living organisms and whether their effectiveness correlates with laboratory testing. This can help to validate the findings and guide future treatment protocols.

Limitations

The limitation of this work was waiting to obtain cases with signs of Avian Colibacillosis which were not certain eventually taking a long period of 3 months and also not having the guarantee of types and ages of birds that would be obtained.

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REFERENCES

Abdelrahman, I.O, Elbagir, N.M., Osman, O.M.A., Sharfi, S.A., Saeed, A.M.A, Musa, H.A., Ashmaig, A.A., Eradaib, I.E. (2008) PCR Detection of *E. coli* in Chicken Fecal Samples. *International Journal of Molecular Medicine and Advanced Sciences*. 4(3), 82-85.

Aberkane, C., Messaï, A., Messaï, C.R. and Boussaada, T. (2023) Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. *Veterinary World* 16(3): 449-454, doi:10.14202/vetworld.2023.449-454.

Article DOI : https://doi.org/10.37284/eajab.7.1.2185

Adebowale, O.; Makanjuola, M.; Bankole, N.; Olasoju, M.; Alamu, A.; Kperegbeyi, E.; Oladejo, O.; Fasanmi, O.; Adeyemo, O.; Fasina, F.O. (2022). Multi-Drug Resistant *Escherichia coli*, Biosecurity and Anti-Microbial Use in Live Bird Markets, Abeokuta, Nigeria. *Antibio tics. 11*, 253. https://doi.org/10.3390/antibiotic s11020253.

Adeite A. (2021). Interesting facts about poultry farming in Nigeria. *https://babbangona.com/5-interesting-facts-about-poultry-farming-in-nigeria.*

Aggad, H., Ahmed, Y., Hammoudi, A. and Kihal, M. (2010). Antimicrobial resistance of *Escherichia coli* isolated from chickens with colibacillosis. *Global Veterinaria* **4** (3): 303-306.

Agyare, C., Etsiapa, B.V., Ngofi, Z.C., Boateng, O.F. (2019) Antibiotic Use in Poultry Production and Its Effects on Bacterial Resistance [Internet]. Antimicrobial Resistance - A Global Threat. IntechOpen. Available from: http://dx.doi.org/10.5772/intechopen.79371.

Ardrey, W.B., Peterson, C.F. & Haggart M. (1968). Experimental colibacillosis the development of carriers in laying hens. *Avian Diseases***12 (3)**: 505-511. PMID: 4879638. DOI:10.2307/1588165 Corpus ID: 29824286

Aworh, M.K., Kwaga, J., Okolocha, E., Harden, L., Hull, D., Hendriksen, R.S., Thakur, S. (2020). Extended-spectrum ß-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. One Health Outlook. 2:8. doi: 10.1186/s42522-020-00014-7. PMID: 33829130; PMCID: PMC7993457.

Aworh, M.K., Kwaga, J., Okolocha, E., Mba, N., Thakur, S. (2019). Prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria. PLoS One. 14(11):e0225379. doi: 10.1371/journal.pone.0225379. PMID: 31751388; PMCID: PMC6872178.

Balouiri. M., Sadiki, M., Ibnsouda, S.K. (2016) Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharm Anal. 6(2):71-79. doi: 10.1016/j.jpha.2015.11.005. Epub 2015 Dec 2. PMID: 29403965; PMCID: PMC5762448.

Casewell, M., Friis, C., Marco, E., McMullin, P. & Phillips, I. (2013). The European ban on growth promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy* **52**: 159-161.

Charlton, B.R. ed. Avian Disease Manual. 6th edition. Athens: American Association of Avian Pathologists (AAAP), 2006.

Das, A., Dhar, P.K., Dutta, A., Jalal, M.S., Ghosh, P., Das, T., Barua, H., Biswas, P.K. (2020). Circulation of oxytetracycline- and ciprofloxacin-resistant commensal *Escherichia coli* strains in broiler chickens and farm environments, Bangladesh. *Vet World*. 13(11):2395- 2400. doi: 10.14202/vetworld.20 20.2395-2400. Epub 2020 Nov 10. PMID: 33363332; PMCID: PMC7750236.

Dawe, J. F. (2014). The relationship between poultry health and food safety. *The Poultry Informed Professional* 77: 1-7.

Fairchild, A.S.; Smith, J.L.; Idris, U.; Lu, J.; Sanchez, S.; Purvis, L.B.; Hofacre, C.; Lee, M.D. (2005). Effects of Orally Administered Tetracycline on the Intestinal Community Structure of Chickens and on *tet* Determinant Carriage by Commensal Bacteria and *Campylobacter jejuni*. *Appl. Environ. Microbiol. 71*, 5865–5872. [Google Scholar] [CrossRef]

Heijnen, L., Medema, G., (2006). Quantitative detection of *E. coli*, *E. coli* O157 and other

Article DOI : https://doi.org/10.37284/eajab.7.1.2185

toxin producing *E. coli* in water samples using a culture method combined with real-time PCR. *Journal of Water Health.* 4:487–498. [PubMed] [Google Scholar] [Ref list]

Kabir, S. M. (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health*, **7**(1), 89–114.

Kowalska-Krochmal, B., Dudek-Wicher, R. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens*. 10(2): 165. doi: 10.3390/pathogens10020165. PMID: 33557078; PMCID: PMC7913839.

Mayers, D.L., Lerner S.A., Ouelette M. (2009). Antimicrobial Drug Resistance: Clinical and Epidemiological Aspects. *Springer Dordrecht Heidelberg; London:* vol. 2. pp. 681–1347. [Google Scholar]

McCormick, J.R. & Johnson, S. (2013). "Biosynthesis of the Tetracyclines. V. Naphthacenic Precursors". *Journal of the American Chemical Society* **85** (11): 1692–1694. doi:10.1021/ja00894a037.

Moreno, M.A., García-Soto, S., Hernández, M., Bárcena, C., Rodríguez-Lázaro, D., Ugarte-Ruíz, M., Domínguez, L. (2019). Day-old chicks are a source of antimicrobial resistant bacteria for laying hen farms. *Vet. Microbiol.* , 230, 221–227. [Google Scholar] [CrossRef].

Nolan, L.K. (2013). *Diseases of Poultry*. 13th ed. Ch. 18. Ames: Wiley-Blackwell, Print. Colibacillosis. [Google Scholar].

Nolan, L.K., Barnes, H.J., Abdul-Aziz, T., Logue, C.M., Vaillancourt, J-P. (2015). Colibacillosis. In: Brugere-Picoux, J., Vaillancourt J.P., Shivaprasasd, H.L., Venne, D., Bouzouaia, M., Association francaise pour l'avancement des sciences (AFAS), editors. *Manual of Poultry Diseases. China: Toppan Printing Leefung.* pp. 301–315. [Google Scholar].

Ojo, O.E., Ogunyinka, O.O.G., Agbaje, M., Okuboye, J.O., Kehinde, O.O., Oyekunle, M.A. (2012). Antibiogram of Enterobacteriaceae isolated from free-range chickens isolated from free-range chickens in Abeokuta, Nigeria. *Veterinary Archives*, 82(6): 577-589.

Okorafor, O.N., Anyanwu, M.U., Nwafor, E.O., Anosa, G.N., Udegbunam, R.I. (2019)**.** Multidrug-resistant enterobacteria colonize commercial day-old broiler chicks in Nigeria. *Veterinary World*, *12*, 418–423. [Google Scholar] [CrossRef]

Oluwasile, B., Agbaje, M., Ojo, O., Dipeolu, M. (2014). Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto Journal of Veterinary Science*; 12:45. doi: 10.4314/sokjvs .v12i1.7. [CrossRef] [Google Scholar].

Pereira, A., Sidjabat, H.E., Davis, S., Vong da Silva, P.G., Alves, A., Dos Santos, C., Jong, J.B.d.C., da Conceição, F., Felipe, N.d.J., Ximenes, A. *et al.* (2024). Prevalence of Antimicrobial Resistance in *Escherichia coli* and *Salmonella* Species Isolates from Chickens in Live Bird Markets and Boot Swabs from Layer Farms in Timor- Leste. *Antibiotics*, 13, 120. https://doi.org/10.3390/antibiotics13020120

Pokrant, E., Vargas, M.B., Navarrete, M.J., Yévenes, K., Trincado, L., Cortés, P., Maddaleno, A., Lapierre, L., Cornejo, J. (2023). Assessing the Effect of Oxytetracycline on the Selection of Resistant *Escherichia coli* in Treated and Untreated Broiler Chickens. *Antibiotics* , 1652. https://doi.org/10.3390/antibiotics12121652

Rahimi, M. (2013). Antibiotic resistance profile of avian pathogenic *Escherichia coli* isolates

Article DOI : https://doi.org/10.37284/eajab.7.1.2185

recovered from broiler chicken farms with colibacillosis in Kermanshah province, Iran. *Global Veterinaria*, 10 (4): 447-452.

Saberfar, E., Pourakbari, B., Chabokdavan, K. & Taj Dolatshahi, F. (2008). Antimicrobial Susceptibility of *Escherichia coli*Isolated from Iranian BroilerChicken Flocks 2005-2006. *The Journal of Applied Poultry Research*, 17 (2):302-304 DOI: 10.33/japr.2007-00102.

