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Molecular prevalence and resistance profile of *Escherichia coli* from poultry in Niger State, Nigeria

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ABSTRACT

Globally, *Escherichia coli* (*E. coli*) are an important cause of foodborne illness and a public health threat, especially in sub-Saharan Africa, including Nigeria. This study aimed to determine the prevalence and resistance of *E. coli* to commonly used antibiotics in poultry farms in Niger State. A total of 164 samples were collected, consisting of 82 each of fresh and fecal dust from visited farms. *E. coli* was isolated by culture, characterized using biochemical methods, and molecular confirmation was done using polymerase chain reaction (PCR). Subsequently, confirmed isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method. The total prevalence of 6.1% (10/164) was obtained by PCR. Dust samples 12.2% (10/82) have a statistically significant (p<0.001) higher prevalence as compared to fecal samples with no *E. coli* detection at all. Notably, all isolates were 100.0 % resistant to ampicillin and amoxicillin-clavulanic acid. A high rate of resistance was found to tetracycline (90.0%), trimethoprim (70 %), sulphonamide (70 %), nalidixic acid (50.0 %) and moderate resistance to ciprofloxacin (40.0%), chloramphenicol (40.0%) and gentamicin (40.0%). Remarkably, 70 % of the isolates were multidrug resistant and also, 20 % of the isolates were pan-drug resistant (PDR). Antimicrobial stewardship from relevant authorities and farmers, especially in combination with educational activities and public awareness campaigns, will help reduce the increasing trend of antimicrobial resistance.

Keywords: Prevalence; resistance; *E. coli*; poultry; Nigeria

INTRODUCTION

Poultry is a good source of high-quality animal protein for the growing population of Nigeria and a vital part of the Nigerian economy, providing smallholder farmers a source of income and livelihood (Awoyomi *et al*., 2022). It accounts for approximately 25% of local meat from livestock produced in Nigeria (Rekwot *et al*., 2018). Notably, the livestock sub-

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sector plays a crucial role in Nigeria's socio-economic development and nutritional security, contributing approximately 37% of the total protein intake for Nigerians (Adeyonu *et al*., 2021). Additionally, poultry are gaining market share, challenging the dominance of red meat. Factors driving this shift include fast food expansion, consumer preferences, competitiveness, and health concerns related to red meat safety (Adeyonu *et al*., 2021).

Antimicrobial resistance (AMR) is now widely acknowledged on a global scale due to the rise of multidrug-resistant organisms, resulting in higher rates of mortality and economic pressure, and Nigeria also faces significant challenges related to AMR (Aworh *et al*., 2019). Antimicrobials are commonly used in veterinary medicine as part of a response to colibacillosis infection (Moulin *et al*., 2008). The indiscriminate use of antimicrobials for production and preventive purposes is recognized to increase the risk of resistance among *E. coli* and other enteric pathogens in Nigeria (Mahmudul Hassan, 2021). Report has shown direct transmission of *E. coli* resistant to streptomycin, sulphonamides and tetracycline from poultry to poultry attendants in Nigeria (Ojeniyi, 1989).

Globally, the effect of *E. coli* in poultry production can be severe with most human infections occurring from contaminated food products of poultry origin (Hamisi *et al*., 2014). It is a public health threat in sub-Saharan African countries, including Nigeria. *Escherichia coli*, a Gram-negative non-spore forming bacterium belonging to the family *Enterobacteriaceae* is a member of the normal microbiota in poultry intestine but causes disease called colibacillosis, with effects such as septicaemia, pericarditis, and death of birds (Guabiraba & Schouler, 2015). Some drug-resistant *E. coli* strains, which may not directly cause disease, remain significant in public health as a reservoir of drug-resistant genes that can be transferred to humans (Aworh *et al*., 2019b). While poultry may serve as a notable reservoir of resistant bacteria in food animals, there is limited data on the multidrug resistance profile of *E. coli* in poultry in the study location. This research aimed to identify *E. coli* from various poultry breeds in Niger State and assess their susceptibility to commonly use antibiotics as well as the existence of multidrug resistance.

METHODOLOGY

Study Area

The research was carried out in Minna, the principal city of Niger State, Nigeria. Niger State spans an area of 76,363 square kilometers, making it the largest state in Nigeria in terms of land area. Niger State is situated in the middle belt and it shares an international boundary with the Benin Republic to the east and has national borders to the north by Zamfara State, west by Kebbi State, south by Kogi State, southwest by Kwara State, northeast By Kaduna State and southeast by Federal Capital Territory, Abuja (Figure 1) (*About Niger - Niger State Official Website*, n.d.). Based on GPS coordinates, Minna is situated approximately at 9°35' North and longitude 6°32' East, with an elevation of 299 meters above sea level. Minna falls within the southern Guinea savannah vegetation zone of Nigeria and covers an area of approximately 490 hectares. The population of Minna as at 2012 was 613,246 (NPC, 2012). The city consists of two Local Government Areas (LGAs), namely Chanchaga and Bosso LGAs in Niger State. Minna comprises twenty-five neighborhoods spread across these two LGAs.

Figure 1: Image of study area with the neighboring states

Sample and Sampling Procedure

A cross-sectional study design was employed, with a sampling frame of different production systems within the study area to ensure a comprehensive and real-time assessment of *E. coli* in Minna, Niger State. A random sampling technique was used by assigning random numbers to the list of farms to select the farms considered in this study. A total of forty-three (43) farms was sampled in Chanchanga and Bosso Local government areas of Niger State. This approach allowed for capturing unique dynamics within various areas in the state. Briefly, with a gloved hand, fecal and dust samples were collected from apparently healthy birds and placed into a sterile sample bottle after obtaining verbal consent from the farm owners. Samples were adequately labeled and transported on ice packs to the bacterial zoonosis laboratory of the Department of Veterinary Public Health and Preventive Medicine, Usmanu Danfodiyo University, Sokoto, for immediate analysis.

Cultural Isolation of *E. coli*

Collected samples were aseptically placed into buffered peptone water (Oxoid, UK) and incubated aerobically at 37°C for 24 hours. A loopful of the broth culture was then streaked onto MacConkey agar (Oxoid, UK) and incubated at 37°C for 24-72 hours. Lactose-fermenting (pink) colonies were transferred to eosin methylene blue (EMB) agar (Oxoid, UK). Colonies displaying a green metallic sheen were considered presumptive *E. coli* and transferred onto a nutrient agar slant bottle for further processing.

Biochemical Characterization of *E. coli*

Presumptive *E. coli* isolates were subjected to biochemical identification using the Indole reaction, Methyl red test, Voges-Proskauer test, and Citrate utilization test (IMViC), as described by Cheesbrough (2006).

Molecular Confirmation of *E. coli*

Briefly, 2-3 colonies of *E. coli* were transferred to 200 µL of nuclease-free water and boiled for 10 minutes to disrupt the cells to release the DNA, followed by centrifugation at 10,000 rpm for five minutes. The supernatant containing DNA was then stored at -20°C until used for polymerase chain reaction (PCR). Confirmation of *E. coli* was done by PCR amplification of the β-d-glucuronidase (*uidA*) gene using forward primers 5'GCGTCTGTTGACTGGCAGGTGGTGG3' and reverse primers 5'GTTGCCCGCTTCGAAACCAATGCCT3' (Gó Mez-Duarte *et al.* 2010). PCR amplification was performed in a final volume of 25 µl reaction mixture containing 12.5 µl of ThermoFisher Master Mix, 0.5 µl each of the forward and backward primers, and 10.5 µl of nuclease-free water (Invitrogen, Carlsbad, CA). The amplification cycles consisted of an initial denaturation at 94°C for 2 min, followed by 40 cycles of 92°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA). PCR products were separated on a 1.5% (w/v) agarose gel in Tris Borate EDTA buffer (pH 8.2), stained with ethidium bromide (10 µg/ml), and visualized with a GelDoc Go Imaging System (BioRad Laboratories, Inc., USA).

Antibiotic Susceptibility Testing

Confirmed *E. coli* isolates were tested for susceptibility to ten of the most important antimicrobials used in the field. This includes ampicillin (10 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (30 µg), kanamycin (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), sulphonamides (300 µg), tetracycline (30 µg), chloramphenicol (30 µg), and trimethoprim (5 µg). Susceptibility testing was done using the Kirby–Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2016). Inhibition zone data were entered into WHONET version 5.6, configured with the tested antimicrobials. Isolates were categorized as sensitive, intermediate, or resistant using CLSI clinical breakpoints and CLSI guidelines for disc diffusion (Clinical and Laboratory Standards Institute 2020). Resistance to at least one agent in three or more antimicrobials of different categories was considered multidrug resistance (MDR) according to Magiorakos *et al.* (2012).

Data and Statistical Analysis

Data were entered into Microsoft Excel 2016 and later exported to SPSS version 16 for inferential statistics. The chi-square test was used to check for associations between the prevalence of *E. coli* and other categorical variables. A p-value < 0.05 was considered significant at a 95% confidence interval.

RESULTS AND DISCUSSION

Cultural Identification of *E. coli* from Poultry Farms

As shown in Table 1, Out of the 164 samples collected, 67 (40.9%) exhibited characteristic pinkish colonies, indicating lactose fermentation, on MacConkey agar (Figure 2), while 22 samples out of 67 (13.4%) displayed the typical green metallic sheen on EMB agar (Figure 3). Following cultural isolation ten of these isolates were phenotypically presumed *E.coli* after biochemical characterization (Figure 4).

Table 1: Sample processing workflow for the detection of *E. coli* in poultry farms in Niger State

Methods	No of samples			No of Positive			Percent
	Faecal	Dust	Total	Faecal	Dust	Total	
Culture	82	82	164	2	20	22	13.4
Biochemical	2	20	22	0	10	10	6.1
PCR	0	10	10	0	10	10	6.1

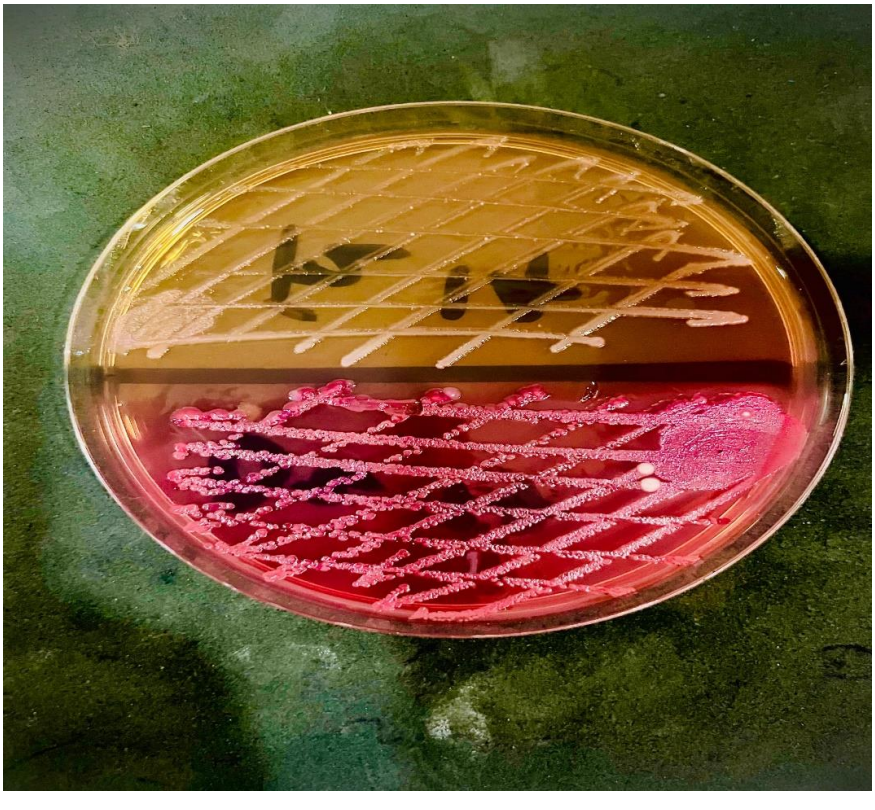

Figure 2: MacConkey agar inoculated with the samples demonstrating growth of lactose fermenters with pinkish colonies

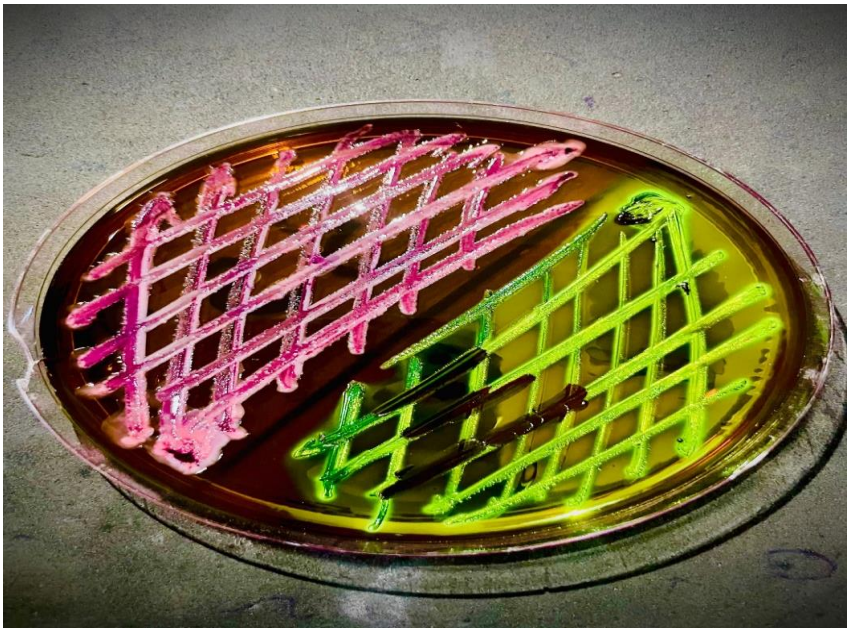

Figure 3: EMB agar inoculated with the lactose fermenters growth from MacConkey agar demonstrating growth with green–metallic sheen colonies

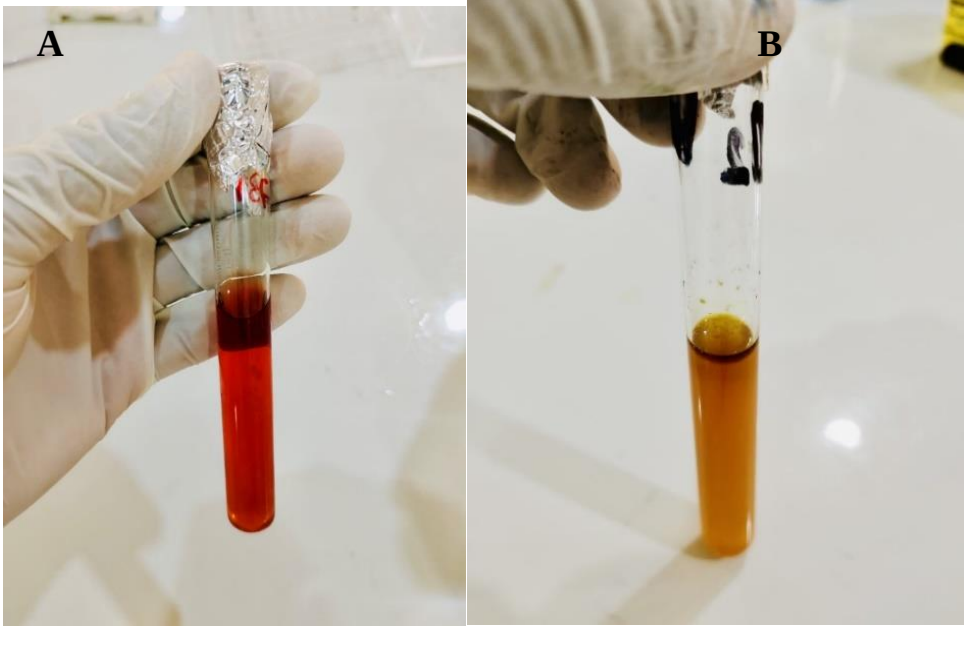

Figure 4: A; Methyl red colour change using methyl red Voges-Proskauer test. B; Indole reaction indicating the red ring in test tube.

Molecular Detection of *E. coli* in Poultry

Following PCR amplification of presumed isolates using the *uidA* gene (Figure 5), the overall detection rate was found to be 6.1 % of *E. coli* from poultry farms in Bosso and Chanchaga local government areas of Niger states. Chanchaga had the highest detection rate of *E. coli* in poultry farms (7.0 %), while Bosso had 4.7 %. However, this difference was found not to be statistically significant (Table 2). This study reported a molecular prevalence of 6.1% of *E. coli* from commercial poultry farms in Niger State, Nigeria. This prevalence is somewhat lower compared with what was obtained (12.1%) by Abubakar *et al*. (2023) in Gusau, Zamfara State. Similarly, other studies have reported the higher prevalence of *E. coli* in poultry. Studies conducted by Ejeh *et al*. (2017) reported a prevalence of 28% in Zaria, while in Kano, 80% prevalence was reported (Ibrahim & Habibu, 2021). In Tanzania, Rugumisa *et al*. (2016) reported 81% prevalence. The lower prevalence in this study could be due to factors like seasonal variation, sample size, and hygienic practices in the farm. A study conducted by Majhi *et al*. (2018) showed that there is seasonal variation in the prevalence of *E. coli* in poultry. This study only detected *E. coli* in dust samples, aligning with findings from studies that *E. coli* and other bacteria can persist in environmental dust reservoirs within animal housing. This persistence is partly due to factors such as dust's capacity to retain microorganisms, including antibiotic-resistant strains, for extended periods. In a study by Schulz *et al*. (2016), it was found out that *E. coli* could survive a long time in dust samples due to the desiccated environment, which reduces the bacteria's metabolic activity and prolongs its viability.

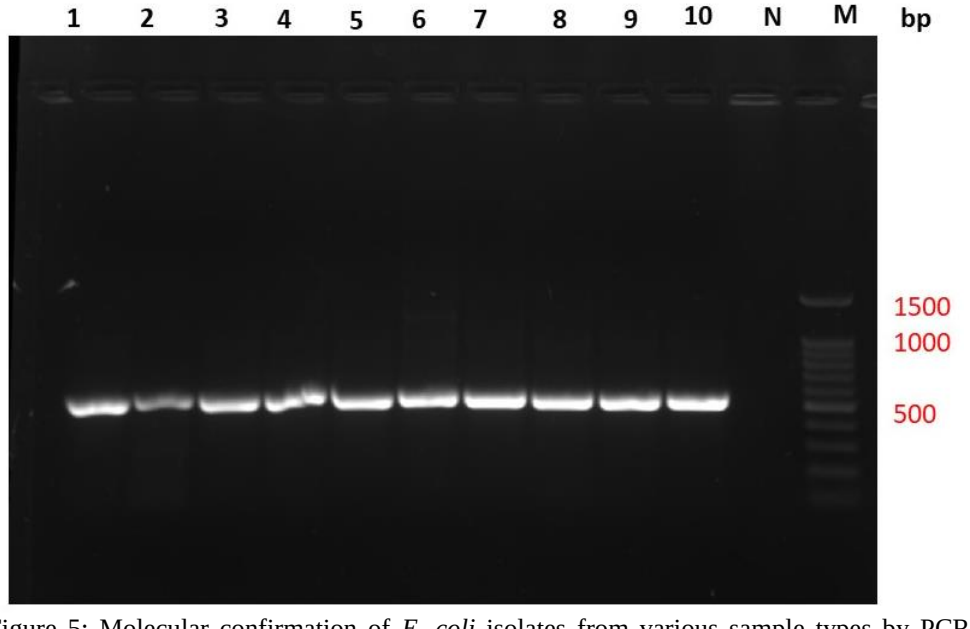

Figure 5: Molecular confirmation of *E. coli* isolates from various sample types by PCR detection of *uidA* gene. The product yielded 503 bp typical of *E. coli* lane 1-10, N: negative control (nuclease free water), M: 100bp DNA ladder (Trans-gene Biotech China)

Table 2: Parameter specific prevalence of *E. coli* infection from poultry farms in Niger State

Parameters	Number of samples	Number of positive	Percent	
Sample Location				χ 0.36, p 0.546
Bosso	64	3	4.7	
Chanchanga	100	7	7.0	
Sample Type				χ 10.6, p 0.001
Dust	82	10	12.2	
Faecal	82	0	0	
Production System				χ 0.67, p 0.412
Deep litter	112	8	7.1	
Battery cage	52	2	3.8	
Chicken Type				χ 1.06, p 0.588
Broilers	84	4	4.8	
Layers	50	3	6.0	
Noilers	30	3	10.0	

Resistance Profiling of *E. coli*

Out of all the confirmed *E. coli* from poultry farms, a high rate of resistance was found to tetracycline (90.0%), trimethoprim (70 %), sulphonamide (70 %), nalidixic acid (50.0 %) and moderate resistance to ciprofloxacin (40.0%), chloramphenicol (40.0%) and gentamicin (40.0%) as shown in Table 3.

Table 3: Antimicrobial susceptibility testing of confirmed *E. coli* from poultry in Niger State

Antimicrobial Class	Clinical Breakpoints	Number of Susceptible (%)	Number of Intermediate (%)	Number of Resistance (%)
Aminoglycosides				
Gentamycin	13 – 14	6 (60)	0 (0)	4 (40)
Kanamycin	14 – 17	3 (30)	5 (50)	2 (10)
Quinolones				
Ciprofloxacin	22 – 25	5 (50)	1 (10)	4 (40)
Nalidixic acid	14 – 18	3 (30)	2 (20)	5 (50)
Folate inhibitors				
Trimethoprim	11 – 15	3 (30)	0 (0)	7 (70)
Sulphonamides	13 – 16	3 (30)	0 (0)	7 (70)
Phenicols				
Chloramphenicol	13 – 17	5 (50)	1 (10)	4 (40)
Penicillins				
Ampicillin	14 – 16	0 (0)	0 (0)	10 (100)
Tetracyclines				
Tetracycline	12 – 14	1 (10)	0 (0)	9 (90)
Beta–lactamase inhibitors				
Amoxycillin clavulanic acid	14 – 17	0 (0)	0 (0)	10 (100)

Notably, all isolates were resistant to ampicillin and amoxicillin-clavulanic acid. Remarkably, 70 % of the isolates were multidrug-resistant (resistant to more than 3 classes of antibiotics) and also, 20 % of the isolates were pan-resistant or PDR (resistant to all antimicrobial agents used in this study) (Table 4). Notably, this study reported a high rate of resistance of *E. coli* to tetracycline, trimethoprim, and sulphonamide. This is likely due to the long-term and extensive use of these antimicrobials in which overuse and misuse of these antibiotics are major drivers. The presence of these antimicrobials resistance has been recorded in previous studies such as (Agusi *et al*., 2024; Al Azad *et al*., 2019; Dehkordi *et al*., 2014; Momtaz *et al*., 2012). Also, the use of these antimicrobials as growth promoters especially tetracycline as stated by Jamal *et al*. (2017) is controversial because it has potential to transfer anti-microbial resistance from livestock to humans.

Table 4: Resistant profiling of confirmed *E. coli* from poultry in Niger State

S/N	Profile	n	PDR	MDR
1	AMC+AMP+CHL+CIP+GEN+KNC+NAL+SUL+TET+TMP	2	2	2
2	AMC+AMP+CIP+GEN+NAL+SUL+TET+TMP	2	0	2
3	AMC+AMP+CHL+NAL+SUL+TET+TMP	1	0	1
4	AMC+AMP+CHL+SUL+TET+TMP	1	0	1
5	AMC+AMP+SUL+TET+TMP	1	0	1
6	AMC+AMP+TET	2	0	0
7	AMC+AMP	1	0	0
	Total	10	2	7

KEY:
PDR=pandrug resistant
MDR= multidrug resistant
AMC = Amoxycillin clavulanic acid
AMP = Ampicillin
CHL = Chloramphenicol
CIP = Ciprofloxacin
GEN = Gentamycin
KNC = Kanamycin
NAL = Nalidixic acid
SUL = Sulphonamide
TET = Tetracycline
TMP = Trimethoprim

Interestingly, high sensitivity to gentamicin was observed in this study. The high sensitivity to gentamicin needs to be investigated, as this present study included few presumptive isolates for susceptibility testing. Probably this could be due to the low sample size in our case. The report has shown the high usage of gentamicin in poultry production in Nigeria (Jibril *et al*., 2021).

Worrisomely, 20 % of the isolates were pan-resistant, showing resistance to the entire antibiotic tested in this study. In addition, 70% were MDR. Studies reveal widespread antimicrobial resistance (AMR) among *E. coli* isolates in poultry, which aligns with current findings of multidrug-resistant (MDR) strains. Research by Davis *et al*. (2018) also supports the high prevalence of resistance, with significant findings that 48% of *E. coli* isolates from retail poultry exhibited MDR. In a 2019 review, Roth *et al*. (2019), emphasized that *E. coli* in broilers globally show significant resistance due to extensive antibiotic usage in poultry production, leading to multidrug and even pan-resistant strains. PDR strains are even more alarming, as they are resistant to all antibiotics tested, leaving very limited treatment options

if these bacteria are transmitted to humans (World Health Organization, 2020). Worrisomely, resistance to antibiotics that are critically important to human medicine were identified (World Health Organization, 2017). This will lead to difficulty in the treatment of previously treatable diseases which can gradually render these drugs less useful and lead to deterioration of the overall health of both animals and humans and a drastic reduction in life span.

CONCLUSION

Taken together, in this study, we observed a prevalence of 6.1 % of *E. coli* in commercial poultry farms in Niger State, Nigeria. Most of the isolates showed high resistance to ampicillin, amoxicillin clavulanic acid, tetracycline, trimethoprim and sulphonamides. Likewise, most of the isolates were multidrug resistant. A moderate number of isolates appear to be pan-resistant (resistant to all antimicrobial agents that were used in this study). Further studies should be done to determine the resistance genes to remaining antimicrobials observed in the *E. coli* isolates. There should be public awareness and regulations by relevant agencies like the Veterinary Council of Nigeria (VCN), Nigerian Veterinary Medical Association (NVMA), and the Federal Ministry of Livestock Development on how to appropriately give antibiotics and other drugs to chickens and humans to avoid antimicrobial resistance**.**

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