Isolation and Characterization of Multidrug-resistant *Escherichia coli* from Poultry Litter samples from Selected Farms in Kano metropolis, Nigeria

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Abstract: *Escherichia coli* is one of the major bacterial entropathogens of public health concerns that cause food-borne diseases, thereby contributing to increased human morbidity and mortality. This study was aimed at isolating and characterizing multidrug-resistant *E. coli* isolates from both local and agric litter samples from selected poultry farms in Kano state. Questionnaire was administered to obtain information such as the size of the poultry farm, types of birds and mode of litter disposal from poultry farmers. A total of 10 samples of litter were aseptically collected 5 each from agric and local poultry farms. Bacteriological investigation on 10 isolates from local and broiler chicken litters for occurrence of *E. coli was* carried out by isolation through culture and identification using biochemical techniques. Antibiotic susceptibility testing was performed using the disc diffusion method. In broiler farms, four out of five (80%) of participating farmers gave antibiotics for prophylaxis. The prevalence of *E. coli* was 90%. All *E. coli* isolates were multidrug resistant. The highest frequencies of resistance by *E. coli* were recorded for septrin (Co-trimoxazole), amoxicillin and chloramphenicol (90-100%).The presence of multidrug resistance was exhibited by all *E. coli* isolates (MAR. index; 0.6-0.9) which may be a high use of antimicrobials in poultry farms. Contamination of chicken litter may be an underestimated source of antimicrobial resistance (AMR) transmission towards animals, humans and the environment with multidrug resistant *E. coli.* Therefore, continued surveillance in chicken litter proliferation as local manure would enable monitoring of AMR risks and trends.

Keywords: antimicrobial resistance, *E. coli*, multidrug resistant, poultry litter

INTRODUCTION

The poultry sector is among the fastest growing agro-based industries worldwide due to increasing demand for egg and meat products (Bolan *et al.,* 2010). Chicken litter is the waste generated in the large quantity in the process (Aires, 2009). This waste is potentially important for land application as an organic fertilizer because of its relatively high nutrient content (Sanchuki *et al.,* 2011). The key safety concerns of chicken litter are its contamination with pathogens, including bacteria, fungi, helminthes, parasitic protozoa, and viruses; antibiotics and antibiotic-resistant genes; growth hormones such `as egg and meat boosters; heavy metals; and pesticides (Oliveira, 2012) Disease burden has however, remained a great challenge in poultry production (Bolan *et al.,* 2010). Some of the common microbial pathogens isolated from fresh poultry litter include *Salmonella* sp*., Campylobacter* spp. and *Escherichia coli* (Aires 2009; Viegas *et al.,*2012; Ejeh *et al.,* 2017; Ngogang *et al.,* 2021). Failure to manage these pathogens in poultry litter has led to various food-borne disease outbreaks in countries such as Bangladesh, Nigeria and Cameroon (Khan *et al.*, 2014; Ejeh *et al.,* 2017;Ngogang *et al.,* 2020) respectively. Antibiotic usage is considered as one of the most important factors in promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Hochmuth *et al.,* 2016). Antibiotic usage selects for resistance in pathogenic bacteria and the endogenous bacterial flora of exposed animals and humans (Lie *et al.,* 2019). This is partly because some poultry farmers are using antibiotics as growth promoters, which are perceived as an inexpensive management practice (William *et al.,* 2012), while other farmers use antibiotics in disease prevention as a mitigation measure against the highly prevalent unhygienic conditions and absence of biosecurity. Consequently, antibiotics are found in litter as residues and bacteria are continuously being exposed to them with a risk of developing resistance.

The extensive use of antibiotics in poultry as growth promoters and most importantly for the control and treatment of diseases have been attributed as the cause of the emergence of bacteria with multidrug resistance associated with poultry. The emergence of resistance has the potential to impact on the treatment and management of infectious diseases in both animals and humans (Mamza *et al.,* 2010).Therefore, the objectives of this study were to isolate and characterize multidrug-resistant *E. coli* from poultry litter samples from selected farms in Kano metropolis.

MATERIALS AND METHODS

Sample collection

A total of 10 poultry litter samples were collected from five selected broilers (agric) and local chickens' farms in Kano Metropolis. Samples collected from each of these poultries were dry feces from the open fields close to the poultry cages. Using sterile gloves, litter was mixed and samples were collected in sterile wide mouthed-containers. After collection the samples were labelled and placed in a sterile plastic containers and were placed in a cooler containing ice packs prior to the transfer within four hours to the laboratory of the Department of Microbiology, Bayero University Kano for further analyses.

Microbiological Assays

Isolation and identification of *E. coli* was done by standard bacteriological methods. MacConkey and EMB agar were used for culturing of specimen and colonies suspected to be *E. coli* were identified by standard methods (Gonzales and Blanco, 1989). Pre-enrichment suspension was obtained by adding 25mg of poultry litter into 225 ml of buffered peptone water, which were incubated at 37^{o}C for 24 h. Isolation of *E. coli* spp. was done by plating pre-enrichment suspension on MacConkey agar followed by incubation as reported by Ngogang *et al.* (2020).

Characterization of the isolated *E. coli*

The conventional biochemical tests carried were; Indole test, Methyl-red test, Voges-proskauer test, Citrate utilization test Motility test using nutrient broth, nutrient agar, EMB agar and SS agar accordingly and Gram staining were carried out (Gyles, 2008).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done using the disc diffusion method as described in Miles (2008). Microbial isolates were grown in nutrient broth for 24 hours and 0.5 Mc Farland standard was prepared to compare the turbidity. Freshly prepared Mueller-Hinton agar was inoculated with the standardized inoculum using sterile cotton swabs. The plates were covered and allowed to dry. Commercially available Antibiotic impregnated filter paper discs were placed on the surface of the agar and the plates were incubated at 37^{o}C for 24 hours. Inhibition zones were measured to the nearest millimeter, the zones were indicated by a lack of microbial growth due to inhibitory concentrations of antibiotics. The inhibitions were read using a Vernier caliper. CLSI standards (2015) were used to classify susceptibility of the isolates as susceptible (S), intermediate (I) or resistant (R).

RESULTS

Cultural, staining and biochemical characteristics

Cultural colony characteristics showed that *E. coli* produces turbid growth in nutrient broth and smooth white to grayish colony on nutrient agar with peculiar fetid odor, dark with metallic sheen on EMB agar and slight pinkish smooth colonies on SS agar. On Gram staining *E. coli* were found Gram negative short rods and arranged as single, paired or in short chain.

The overall isolation rate of *Escherichia coli* from local and broilers chicken litters was 90%. The isolation rate of *E. coli* from agric (broilers) chicken litters was 80% while from local chicken litters it was 100% (Table 1).

Antimicrobial susceptibility of the bacterial isolates to antibiotics

The standard disc diffusion method as described in Miles (2008) was used for the in vitro determination of the sensitivity to the antimicrobial agents. Ten antibiotics were chosen as shown in table 2.

The results of antibiotic resistance profile have been shown in Table 2. *E. coli* isolates of agric litter samples were highly resistant 100% to septrin, tetracycline, ofloxacin and streptomycin respectively and 90% resistant to chloramphenicol and amoxicillin. *E. coli* isolates of locallitter samples were highly resistant to septrin and streptomycin (100%) followed by chloramphenicol, augmentin and ofloxacin (90%).The lowest were erythromycin, ciprofloxacin tetracycline and gentamycin (40%). Thirty three percent (33%) of *E. coli* isolates were resistant to nine (9) antibiotics, forty four percent (44%) of *E. coli* isolates were resistant to 8 antibiotics and eleven percent (11%) of *E. coli* were each resistant to seven (7) and six (6) antibiotics.

Table 1: Isolation of *Escherichia coli* from local and broilers chicken litters

S/No	Chicken type	Number of processed samples	Number of positive samples
1	Local	5 (50)	5 (100)
2	Broilers	5 (50)	4 (80)
	Total	10 (100)	9 (90)

Table 2: Resistance pattern of *Escherichia coli* isolates from poultry litter

Isolate	Antibiotic profile	R (%)	I (%)	S (%)	MAR	MDR
L1	SXT,CH,E,AM,AU,TET,OFX,S	8 (80)	2 (20)	0 (0)	0.8	+
L2	SXT,CH,AM,AU,PES,OFX,S	7 (70)	2 (20)	1 (10)	0.7	+
L3	SXT,AM,AU,TET,OFX,S	6 (60)	1 (10)	3 (30)	0.6	+
L4	SXT,CH,SP,CPX,,AM,AU,CN,S	8 (80)	1 (10)	1 (10)	0.8	+
L5	SXT,CH,E,CPX,AM,AU,CN,OFX,S	9 (90)	0 (0)	1 (10)	0.9	+
A1	SXT,E,AM,AU,CN,TET,OFX,S	8 (80)	0 (0)	2 (20)	0.8	+
A3	SXT,CH,E,AM,AU,CN,TET,OFX,S	9 (90)	1 (10)	0 (0)	0.9	+
A4	SXT,E,CH,AM,AU,CN,TET,OFX,S	9 (90)	1 (10)	0 (0)	0.9	+
A5	SXT,CH,E,AM,CN,TET,OFX,S	8 (80)	1 (10)	1 (10)	0.8	+

KEY: SXT = Septrin CH = Chloramphenicol E = Erythromycin
CPX = Ciprofloxacin AM= Amoxicillin AU = Augmentin
CN = Gentamycin TET = Tetracycline OFX = Ofloxacin
S = Streptomycin **R=** Resistant **I** = Intermediate
S = Susceptible **MAR=** Multi antibiotic resistance
MDR= Multi-drug resistance (when the isolate is resistant to more than 3 antibiotics)
L1-L5 = *E. coli* isolates from local poultry litter
A1-A5 = *E. coli* isolates from agric poultry litter

In this study, it was observed that more than 80% of the *E. coli* isolates were resistant to more than three antibiotic. This is consistent with the study by (Moustafa and Mourad, 2015), which provided direct evidence that antimicrobial use in animals selects for antimicrobial-resistant bacteria that may be transferred to humans through food or direct contact with animals. Multidrug resistance to more than two antimicrobial agents was detected in 9 (90.0%) of the isolates. It was observed in this study that multiple antibiotic resistance was common among *E. coli.* These results are in agreement with previous reports in Nigeria (Raji *et al.,* 2007; Olonitola *et al.,* 2015). Salihu *et al.* (2014) further explained that the excessive use of antibiotics in poultry results from being freely available and readily affordable. The resistance observed in *E. coli* isolated from local chickens has been observed to have resulted from the transfer of resistance gene(s) from another host in the same production environment (Salihu *et al.,* 2014).

DISCUSSION

In this study, higher isolation rate 100.0% of *E. coli* from local chickens was observed while from broilers 80.0% was observed. A possible explanation for this difference may be due to the increased use of antibiotics for treatment and as growth promoter in broiler chickens (Nwankwo *et al.,* 2014; Ejeh *et al.,* 2017). The high occurrence of *E. coli* in local chickens may be due to the fact that local chickens in Nigeria are commonly reared under free range conditions with minimal care, as chickens scavenge for food and water (Geidam *et al.,* 2012) hence are exposed to environmental contaminants and bacterial infections. Amadi *et al.*(2015) further explained that local chickens in Nigeria are neither vaccinated nor given any antibiotic medication and their sources of feed include food remains, grasses, maggots from cow dungs and other environmental waste which may expose them to pathogenic bacteria including *E. coli*.

Similar MAR index from both agric (0.8-0.9) and local farms (0.6-0.8) were recorded in this study which may imply similar level of antibiotics usage and sanitary practice. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used (Miranda *et al.,* 2008). Zinnah *et al*. (2008), also states that MAR index values greater than 0.2 indicate existence of isolate from high risk contaminated source with frequency use of antibiotics while values less than or equal to 0.2 show bacteria from source with less antibiotics usage. Higher MAR indices as shown in the results of this work emphasizes need for surveillance and remedial measures which is public health concern as litter is used as a source of manure.

High level of antibiotic resistance of the *E. coli* isolates(100%) to septrin and (80%) chloramphenicol has been identified and this is because heavy metals as well as antibiotics used in animal farming might promote the spread of antibiotics resistance via co-selection (Abdel-Tawab*et al.,* 2015) and resistance to antibiotics can be conferred by chromosomal or mobile genetic elements (e.g. plasmid). These findings are in agreement with that of Romanus *et al.,* (2012) with high prevalence of *Escherichia coli* strains that are resistance to commonly prescribed antibiotics. This was consistent with findings in this study in which it was observed that more than 50% of the *E. coli* isolates showed a MDR pattern, with the highest resistance profile being associated with septrin, streptomycin, amoxicillin, and ofloxacin.

These findings were also consistent with those in previous study conducted in Bangladesh, in which it was also observed that *E. coli* isolates from cattle had high resistance against septrin, streptomycin, amoxicillin, ofloxacin, sulfamethoxazole/trimethoprim, ciprofloxacin, ampicillin and tetracycline (Zinnah *et al.,* 2008).

Another study, (Moustafa and Mourad, 2015) found that the use of antimicrobials in veterinary practice as therapeutic and prophylactic agents, in addition to use as antimicrobial growth promoters, greatly influence the prevalence of resistance in animal bacteria and poses risk for the emergence of antibiotic resistances in human pathogens. (Moustafa and Mourad, 2015) further observed that isolates which are resistant to two or more antibiotics may have originated from high–risk sources of contamination like commercial poultry farms, where antibiotics are commonly used.

CONCLUSION

The finding from this work established that *Escherichia coli* were isolated from poultry litter. Antibiogram of the isolates revealed all the isolates were highly resistant to septrin, chloramphenicol, amoxicillin and streptomycin antibiotics from both the Agric poultry and local poultry litters isolates. The resistant pattern in both isolates is a major public health concern as multidrug resistant *E. coli* present in the poultry litter.

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