Original Article

Antibiotic Sensitivity Pattern and Risk Factor of *Escherichia coli* Isolated from the Poultry Samples Collected in Veterinary Laboratory, Birendranagar, Surkhet

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Abstract

A study was conducted from March to April 2020 at Veterinary Laboratory, Birendranagar, to ascertain the antibiotic sensitivity pattern and associated factors of Escherichia coli isolated from the poultry samples. **Objective:** A total of 50 liver samples were collected and the questionnaire was filled for the corresponding demographic data. The organisms were identified and isolated. Based on the culture characteristics in different agars (nutrient agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar) and the response of the culture toward the biochemical tests, the microorganisms were identified and isolated. **Method:** Institute of Clinical and Laboratory Standards - recommended Kirby - Bauer disc diffusion method - diffusion method performed for antibiotic susceptibility testing on Muller - Hinton agar. The antibiotic disks used in the study were ciprofloxacin (CP), amikacin (AK), enrofloxacin (EX), gentamicin, azithromycin, tetracycline (TE), and amoxicillin (AMX). **Result:** The results showed that 36% of the samples were positive for *E. coli*. Out of 23 birds of age below 28 days, 5 (21.74%) birds were tested positive for *E. coli*. Similarly, out of 27 birds of age above 28 days, 13 (48.15%) birds were tested positive for *E. coli*. The relation between *E. coli* prevalence and the age of the bird was found to be nonsignificant ($P > 0.05$) according to the Pearson Chi square test. Out of 33 samples from the farm with treated water, 13 (39.39%) of the samples were tested positive for *E. coli*. Similarly, out of 17 samples from the farm with untreated water, 5 (41.67%) of the samples were tested positive for *E. coli*. The prevalence of *E. coli* was significantly ($P < 0.05$) dependent upon the water treatment in the farm. There were 41 broiler samples out of which 16 (39.02%) samples were tested positive for *E. coli*. Similarly, there were 9 layer samples, out of which 2 (22.22%) samples were tested positive for the *E. coli*. There was a nonsignificant ($P > 0.05$) relation of the prevalence of *E. coli* with the type of bird. Among the *E. coli* positive samples(36%), the antibiotic sensitivity pattern was 55.56% for AK, 0.00% for AMX, 61.11% for EX, 38.89% for gentamycin, and 22.22% for TE. Among all the antibiotics, CP showed the highest sensitivity (83.33%) against *E. coli*. **Conclusion:** Among all the associated factors, water treatment in the poultry farm showed a significant ($P < 0.05$) effect on the prevalence of *E. coli*. Thus, treatment of the water in the farm is recommended to decrease the chances of *E. coli* prevalence in the farm.

Keywords: *Escherichia coli*, prevalence, resistance, sensitivity

Introduction

Antibiotic resistance is one of the emerging problems in animal medicine and human medicine as well. The sole reason for this emerging problem of antibiotic resistance is the unscientific and haphazard use of antibiotics.[1-5] The use of antibiotics in poultry in Nepal is extensive and unmanaged. Resistance is not only developed by the corresponding bacteria but also developed by the flora of the exposed individual animal.[6-10] The modern commercial poultry feed consists of antibiotics as antimicrobial growth promoters which continuously supply antibiotics to the poultry that increases the risk of antibiotic resistance.

Escherichia coli is one of the common bacteria found in the intestine of poultry. The disease condition that arises due

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to this bacterium is referred to as colibacillosis. That was found in Figures 1-3. Colibacillosis is spread through fecal contamination from the feed and equipment of the farm, also from the hatchery from the contaminated eggs. The unscientific management and poor biosecurity are the major causes of the contagious disease in the poultry of Nepal. Thus, antibiotics are used haphazardly to compensate the ill management and poor biosecurity. This unsystematic use of antibiotic is the principal cause of antibiotic resistance.

Antibiotic sensitivity and resistance are related to the antagonistic approach. Antibiotic sensitivity is the susceptibility of the bacteria to antibiotics. In the context of emerging problem of antibiotic resistance, the importance of the antibiotic sensitivity pattern is foremost for the selection of antibiotics.[11-15] In the sensitivity test, the susceptibility of the bacteria to different antibiotics is checked in accordance with the diameter of the zone of inhibition. Moreover, the antibiotic showing better sensitivity against the specific bacteria is selected in the treatment protocol.[16,17]

The use of the antibiotic sensitivity pattern for the selection of antibiotics can be highly beneficial for the effective treatment of the diseases. This can help a farmer to achieve higher production and eventually aid the livestock and poultry economy.[18-21]

Methodology

Study area

The study was conducted from March 2020 at Veterinary Laboratory, Birendranagar, Surkhet. The samples were collected from the broiler chicken brought to the laboratory.

Collection of samples

The samples were collected from the poultry brought to Veterinary Laboratory, Surkhet. With the use of sterilized forceps and scissors, liver samples were collected in sterile Petri plates.

Sampling method

The liver samples were collected randomly from the poultry brought to Veterinary Laboratory for the postmortem examination. Fifty liver samples were collected, out of which 41 samples were collected from broilers and 9 samples were collected from layers.

Processing of sample

First, the liver samples were collected from the poultry in sterile Petri plates using sterile forceps and scissors. A sterile loop was inserted in the liver sample to take out culture material from the collected liver samples. The primary culture of the sample was carried out in nutrient agar and MacConkey agar. The samples with lactose-fermenting colony on MacConkey agar were again cultured in EMB agar. Then, biochemical tests (indole, Methyl Red / Voges-Proskauer (MR/VP), citrate, and oxidase) were performed on the colonies showing metallic sheen. Then, Gram staining was also performed for the confirmation of different biochemical tests. Then, the selected colonies were suspended on saline and vortex tube so that the organisms in the colonies are suspended. Mueller–Hinton agar (MHA) surface was inoculated with the swab from the colony. Complete covering of the MHA plate was done with the swab. The plate was then incubated for 15 min for the standardizing the inoculum suspension. The antibiotic disk was placed on the plate with the help of a disk dispenser. Five disks were placed on a plate.

Isolated colony of *E. coli* was taken in a sterile swab from the EMB agar. The plate was inverted before placing them in incubator and then incubated at 37°C for 18–24 h and then, the zone of inhibition was measured with the special ruler. The zone of inhibition was interpreted as resistance, intermediate, and sensitive according to the Clinical and Laboratory Standard Institute M100 S24 Manuel Standard zone of inhibition.

Antimicrobial disks

The antibiotics used to find the zone of inhibition are mentioned Table 1 and Figure 1 with their concentration.

Data entry and analysis

The data collected from the questionnaire survey and the microbiological examination were entered into MS Excel 360 spreadsheets to form the primary database of the study. Then, the data were imported into IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.. The frequency table was calculated using SPSS. For the qualitative statistical relation between various factors and

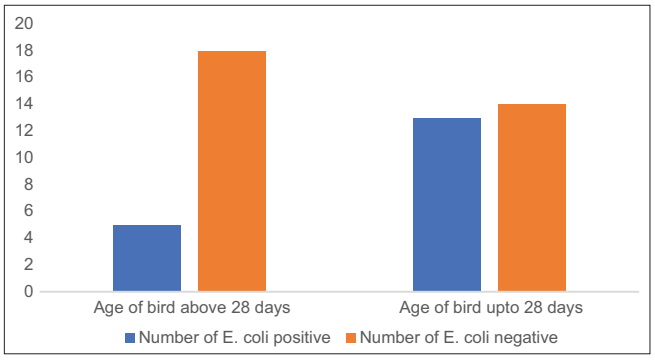

Figure 1: *Escherichia coli* prevalence according to the age of bird

Table 1: Antibiotics with their disk concentration

Antibiotics	Concentration (mcg)
AM	5
AMX	10
AZM	15
CIP	10
Ex	5
GEN	10
TE	30

AM: Amikacin, AMX: Amoxicillin, AZM: Azithromycin, CIP: Ciprofloxacin, Ex: Enrofloxacin, GEN: Gentamicin, TE: Tetracycline

Table 2: Pearson Chi-square test result and interpretation

Factor	Number of *E. coli* positive	Number of *E. coli* negative	*P*	Relation
Age of bird above 28 days	5	18	0.053	Nonsignificant
Age of bird up to 28 days	13	14		
Untreated water	13	20	0.048	Significant
Treated water	5	12		
Broilers	16	25	0.342	Nonsignificant
Layers	2	7		

E. coli: *Escherichia coli*

Table 3: Sensitivity pattern of different antibiotics against *Escherichia coli*

Drug	Sensitive, *n* (%)	Intermediate, *n* (%)	Resistance, *n* (%)	Total
AM	10 (55.56)	5 (27.78)	3 (16.67)	18
AMX	0	0	18 (100.00)	18
AZM	12 (66.67)	3 (16.67)	3 (16.67)	18
CIP	15 (83.33)	3 (16.67)	0	18
Ex	11 (61.11)	1 (5.56)	6 (33.33)	18
GEN	7 (38.89)	7 (38.89)	4 (22.22)	18
TE	4 (22.22)	5 (27.78)	9 (50.00)	18

AM: Amikacin, AMX: Amoxicillin, AZM: Azithromycin, CIP: Ciprofloxacin, Ex: Enrofloxacin, GEN: Gentamicin, TE: Tetracycline

the prevalence of the *E. coli*, Pearson Chi-square test was run as shown in Tables 2-3. The significance between parameters was tested at $P = 0.053$.

Results

Out of 50 samples collected, *E. coli* isolates were collected from 18 samples. There were 23 samples from the bird of age above 28 days, out of which *E. coli* isolates were collected from 5 samples. There were 27 birds of age up to 28 days, out of which 13 samples were tested for *E. coli* isolates.[22] Thirty-three samples were collected from poultry farms using treated water out, of which *E. coli* isolates were collected from 13 samples. Seventeen samples were collected from poultry farms using untreated water, out of which *E. coli* isolates were collected from 5 samples. Forty-one broiler samples were collected, out of which 16 samples were detected with *E. coli* isolates. Nine layer samples were collected, out of which *E. coli* was confirmed from 2 samples.[23]

Antibiotic susceptibility test (AST) was performed in 18 samples with *E. coli* isolate. Out of 18 samples, amikacin was sensitive in 10 samples, intermediately sensitive in 5 samples, and resistant in 3 samples. For amoxicillin (AMX), all 18 samples were resistant. For azithromycin (AZM), 12 samples were sensitive, 3 samples were intermediately resistant, and 3 samples were resistant.[24,25] For ciprofloxacin (CP), 15 samples were sensitive, 3 samples were intermediately resistant, and none of the samples were resistant. For enrofloxacin, 11 samples were sensitive, 1 sample was intermediately resistant, and 6 samples were resistant. For AZM, 12 samples were sensitive, 3 samples were intermediately resistant, and 3 samples were resistant. For gentamycin, 7 samples were sensitive, 7 samples were intermediately resistant, and 4 samples were resistant. For tetracycline, 4 samples were sensitive, 5 samples were intermediately resistant, and 9 samples were resistant.[26]

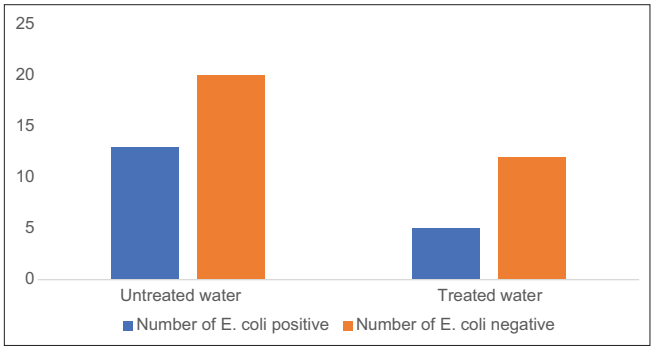

Figure 2: *Escherichia coli* prevalence according to the water treatment in farm

Discussions

The prevalence was reported to be 36% in Surkhet which was higher than the prevalence reported from a study in the same laboratory in 2017 as Sapkota *et al*., 2020, which reported a 31% prevalence of E. coli. However, the prevalence was lower than the report by Siddique *et al*., 2006, in Bangladesh which was 60%, but Dhakal *et al*., 2002; Dhakal *et al*., 2003, reported an 11% prevalence in Padampokhari village of Makawanpur.[27-29]

The prevalence was higher in young age birds of up to 28 days. However, there was a nonsignificant ($P > 0.05$) relation of the *E. coli* prevalence with the age of the bird.

Moreover, the treatment of the water in the poultry farm showed a significant ($P < 0.05$) relation with prevention of E. coli which was in agreement with Sapkota *et al*., 2020. However, the type of bird (broilers or layers) was nonsignificant ($P > 0.05$) for *E. coli* prevalence. The result lucidly directed that the farms not using treated water were at higher risk of *E. coli* prevalence. CP was reported to be the most sensitive antibiotics and AMX was the least sensitive antibiotics against E. coli. Similar results were reported by Sapkota *et al*., 2020, Aggad *et al*., 2010, and Hossain *et al*., 2008.[30-33]

Conclusions

The study reported a 36% prevalence of *E. coli* from the 50 samples.

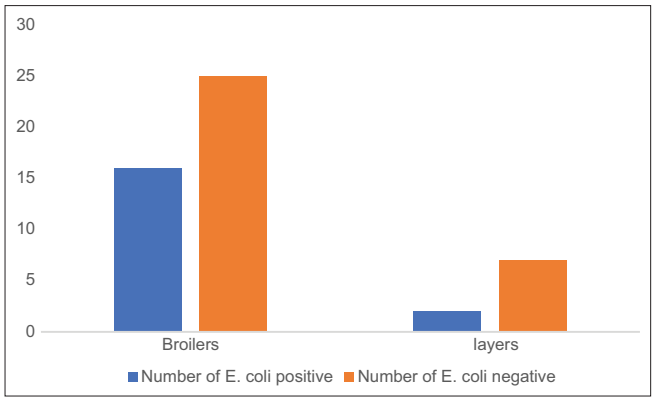

Figure 3: *Escherichia coli* prevalence according to the type of poultry

Most of the factors were statistically nonsignificant ($P > 0.05$) with *E. coli* prevalence. However, the treatment of water in the farm was statistically significant ($P < 0.05$) with the prevalence of *E. coli*. Thus, farmers can be recommended to treat the water in the farm which can significantly decrease the chances of *E. coli* infestation in the farm.

In the case of antibiotics, the recommendation for the choice of antibiotics must be made only after the AST of the isolated *E. coli* from the samples.

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Conflicts of interest

There are no conflicts of interest.

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