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Prevelance and associated risk factor of *escherichia coli* from the cases of poultry at regional veterinary laboratory (RVL) Surkhet, Nepal

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Abstract
A study was conducted at Regional Veterinary Laboratory (RVL), Birendranagar, Surkhet from September 2016 to January 2017 to find out the prevalence and associated risk factor of E.coli from the cases of brought to RVL, Surkhet. Hundred (100) liver samples brought at RVL for postmortem of poultry were collected for the study and questionnaire survey was conducted to take demographic data. Isolation and identification of organisms and were made by examination for cultural characteristics in Nutrient agar, MacConkey agar, EMB agar, Gram's stain and biochemical tests (Indole test (+ve), MR test (+ve), VP test (-ve), Citrate utilization test (-ve) and Catalase test (+ve). The results showed that the prevalence of E. coli in poultry was found to 31% positive. A total of 31 positive cases were kept for antibiotic sensitivity test against six antimicrobials: Amoxycillin (AMX-10 Mcg), Azithromycin (AZM-15 Mcg), Colistin Sulphate (CL-10mcg), Enrofloxacin (EX-5 Mcg), Gentamycin (GEN-10 Mcg) and Tetracycline (TE-30mcg) .The sensitivity test was done by standard disk diffusion method. The overall prevalence of E.coli was found to be 31%. On statistical analysis for the risk factors according to age, source of water and frequency of clean drinker was found to be non- significant ($P > 0.05$) and status of water (treated/untreated) was found to be significant ($P < 0.05$). The antibiotic sensitivity test on the positive case were found to be: Colistin sulphate-96.77%, Azithromicin,-80.64%, Gentamicin-70.96%, Enrofloxicin-38.71%, Tetracyclin-22.58%, Amoxycilin-0% sensitive and Amoxycilin-100%, Tetracyclin-77.42%, Enrofloxicin-61.29%, Gentamicin-25.80%, Azithromicin-19.35%, Colistin sulphate-3.22% resistant respectively. Out of total 100 cases from Surkhet, 75 poultry farmers used tap water and 25 used deep well as a source of water in which, 4 were obtained from deep well and 27 were obtained from tap water source for drinking purpose in poultry were positive. All the risk factors I had considered are statistically non-significant except status of water (treated/un-treated) So, I recommend to provide treated water for the poultry to avoid E.coli contamination and further studies should be done considering other risk factors. Use of antibiotic in poultry is recommended only after scheduled and proper sensitivity analysis.

Keywords: prevalence, *E. coli*, sensitivity, resistance

1. Introduction
Livestock and Poultry sector plays significant role in the Nation's economy. It contributes 11 % in Total Gross Domestic Product and 26.8% in Agricultural Gross Domestic Product (MoAD, 2072/ 073). A total of 31, 991 poultry farmers, 62, 451 local male birds, 32, 917 egg laying hens, 54,586 non-laying hens, 4, 808 daily and 17, 44, 601 annual egg production is achieved with an income of Rs. 5, 96, 02, 062 being generated by selling eggs and milk. Further, a total of 1, 42, 180 poultry are raised and the 1, 35, 071 poultry are supplied for finishing resulting in the total meat production of 3, 10, 663 Kg. The annual production of milk is 17, 34, 000 metric ton, meat is 3, 03, 000 metric ton and egg is 90, 00, 00, 000. (DLSO, Surkhet, 2072/ 073)
The present status of milk, meat and egg consumption in the country per individual per annum is 64 liters, 11 Kg and 32 respectively (DLS, 2072/ 073). The fulfillment of standard requirement of milk, meat and egg being 91-liter, 14 Kg and 48 per individual per annum requires an increase in milk, meat and egg production by 35 %, 25 % and 45 % respectively. Livestock sector provides employment to 15, 000 individuals which is targeted to be increased to 71, 000 by the end of 3 years (CBS, 2072/ 073). With the end of 14th Two Year Plan (2073/

74 - 2075/ 76), Government of Nepal have aimed to increase the employment by 5 times making the country self-reliant in poultry and livestock production. The government has focused on providing technical, financial and other necessary services including commercialization of Livestock and Poultry farming locally to the Livestock and Poultry farmers, community, cooperatives and entrepreneur making the country self-sustainable.

The total meat production of Surkhet district is 4794 metric ton while the meat availability is 13 Kg per individual per annum. The daily supply of chevon, chicken, buff and pork meat in Surkhet district is found to be 875 Kg, 4023 Kg, 1475 Kg and 240 Kg respectively (DLSO, Surkhet, 2072/ 073).

Material and Methods

Site of research

The study was conducted in Regional Veterinary Laboratory, Surkhet.

Collection of samples

Samples were collected from birds taken for the postmortem examination at RVL, Surkhet. First, the poultry is immersed in the solution containing Lysol for 30 sec-1min and prepared for postmortem. The liver sample is collected in sterile Petri plates plastic bags with the help of forceps and scissors immersed in Lysol solution in kidney tray before postmortem started.

Duration of study

Research started from August 2016 to February 2017.

Sample size calculation

$$N = \frac{Z^2_{1-\alpha/2} * p\,(1-p)}{e^2}$$ (Daniel, 1999)

Where, Z= 1.96,

P = expected prevalence

e = maximum tolerable error

Using the previous prevalence 12.2 % (Basnet *et al.*, 2005) and confidence interval 95%, the sample size comes to be 164.5~ 165.

Sampling technique

100 Samples (96 from Broiler, 4 from Layers) were collected from the dead birds that had been registered for postmortem to RVL, Surkhet. The sample was collected randomly from the poultry which were brought for postmortem at RVL, Surkhet.

Sample processing

- Sample collection
- Sterile loop was inserted to the liver sample from the sterilized part by heated spatula and loopful material is taken for the culture
- Thus, obtained sample was cultured on Nutrient agar and MacConkey agar primarily
- Samples with lactose fermenting colony on MacConkey agar was Secondary cultured on the EMB agar
- The colony which show metallic sheen in EMB agar.
- Gram staining and the biochemical tests (Indole, MR, VP, Citrate, Catalase, Oxidase,) for the conformation of isolated sample were done.
- The colonies were suspended on saline and Vortex the tube to make suspension of organism
- Then, adjust the inoculum to a turbidity equivalent to a 0.5 McFarland standard. (corresponds to approximately 1.5 X 108 CFU/ml)
- MHA surface is inoculated with the swab, i.e. the entire surface of the plate is cover
- Then rotate the plate approximately 60° and repeat the swabbing procedure. Rotate the plate 60° again and swab the entire plate a third time
- The plate is incubated for the 15 minutes for standardizing the inoculum suspension.
- Manually the antibiotic disc is applied on the plate 5 disks on a 100 mm plate. Total 10 Antibiotics were applied
- Pure and well isolated colony of E coli from the EMB agar is taken in sterile swab
- The plate with antibiotic disc is incubated with agar side up (i.e. by inverting the disc)
- Incubate in ambient air at 37°C for 18–24 hours.
- Then the Zone of inhibition was measured by a ruler with millimeter
- The Zone of inhibition was interpreted as Resistance Intermediate and Sensitive by Using the CLSI M100 S24 Manuel Standard zone of inhibition

Antimicrobial discs

Following commercially available antimicrobial disc (Himedia, India) were used.

Table 1: Antimicrobial agents with their disc concentration

Antimicrobial	Symbol	Concentration
Enrofloxacin	Ex	5 mcg
Gentamicin	GEN	10mcg
Azithromycin	AZM	15mcg
Tetracycline	TE	30mcg
Colistin Sulphate	CL	10mcg
Amoxicillin	AMX	10mcg

Source: CLSI, 2015

Recording and interpreting results

The zones of growth inhibition were compared with the zone-size interpretative table 5 provided by Clinical and Laboratory Standards Institute (CLSI, 2015). Antimicrobial testing results were recorded as susceptible, intermediate and resistant according to zone diameter interpretive standards provided by (CLSI, 2015).

Table 2: Zone diameter (in mm) interpretive standards for *E. coli*

Parameters	Resistant	Intermediate	Sensitive
Enrofloxacin(Ex,5 mcg)	-	-	30-40
Gentamicin(GEN,10mcg)	12	13-14	15
Azithromycin(AZM,15mcg)	13	14-17	18
Tetracycline(TE,30mcg)	14	15-18	19
Colistin Sulphate(CL,10mcg)	10	-	11
Amoxicillin(AMX,10mcg)	-	-	19-25

(Source: CLSI, 2015)

Data interpretation

Data from microbiological examination and questionnaire survey was inserted into MS-excel spread sheet to create a database. The data were analyzed statistically using Chi-square (χ^2) test (IBM SPSS Statistics version 20.0) and Fisher's Exact Test. Differences between parameters were tested for significance at p-value of 0.05.

Results

Out of 100 dead birds carried out for postmortem in RVL, Surkhet during the study from September 1 to February 1, 31 were found to be positive for the *E.coli* infection in liver samples from different poultry farms of the Surkhet. Along with *E.coli*, other bacteria identified in the growth were *Salmonella* spp., *Klebsiella* spp., *Bacillus* spp., *Staphylococci*, *Streptococci*, and few were unidentified growth.

Out of total 100 cases, 75 poultry farmers used tap water and 25 used deep well as a source of water. Out of the 31 positive cases from Surkhet, 4 were obtained from deep well and 27 were obtained from Tap water source for drinking purpose in poultry. All the positive cases using deep well as water source, were not treated by water sanitizer. But, in positive cases using tap water, 4 were found undergoing water treatment while the rest of 23 didn't used any water sanitizer.

Table 3: Chi square and Fisher's exact test results and interpretation

Risk factors	Number of +ve	Number of –ve	p-value	Remarks
Age <28 weeks	16	42	0.3857	Non-significant
Age>28 weeks	15	27		
Deep well water	4	21	0.061	Non-significant
Tap water	27	48		
Cleaning waterer <2 weeks	12	39	0.099	Non-significant
Cleaning waterer >2 weeks	19	30		
Treated water	4	29	0.004173	Significant
untreated water	27	40		

Table 4: Chi Square test showing relation of source water with treatment

S.N.	Deep Well	Tap Water	P value	Remark
Treated Water	8	25	0.902	Non-Significant
Untreated Water	17	50		

Table 5: Fisher's exact test showing significance of treatment of water

S.N.	Positive	Negative	P value	Remark
Treated Water	4	21	0.011	Significant $P<0.05$
Untreated Water	23	27		

Table 6: Sensitive, intermediate and resistance in different antibiotics

Drug	Sensitive		Intermediate		Resistance (%)		Total
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	
Amoxicillin	0	0	0	0	31	100	31
Azithromycin	25	80.64	0	0	6	19.35	31
Colistin Sulphate	30	96.77	0	0	1	3.22	31
Enrofloxacin	12	38.70	0	0	19	61.29	31
Gentamicin	22	70.96	1	3.22	8	25.80	31
Tetracycline	7	22.58	0	0	24	77.41	31

Overall prevalence

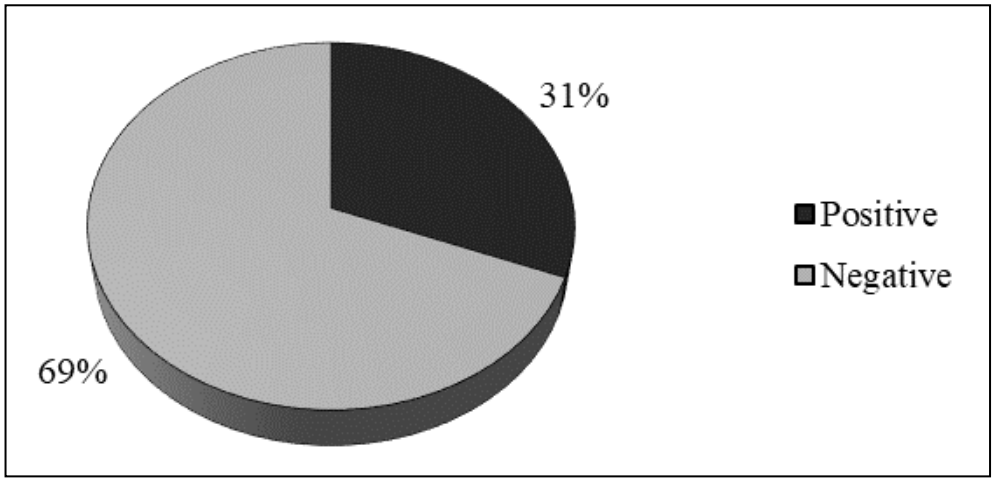

Fig 1: Pie chart showing overall prevalence of *E. coli* in Surkhet

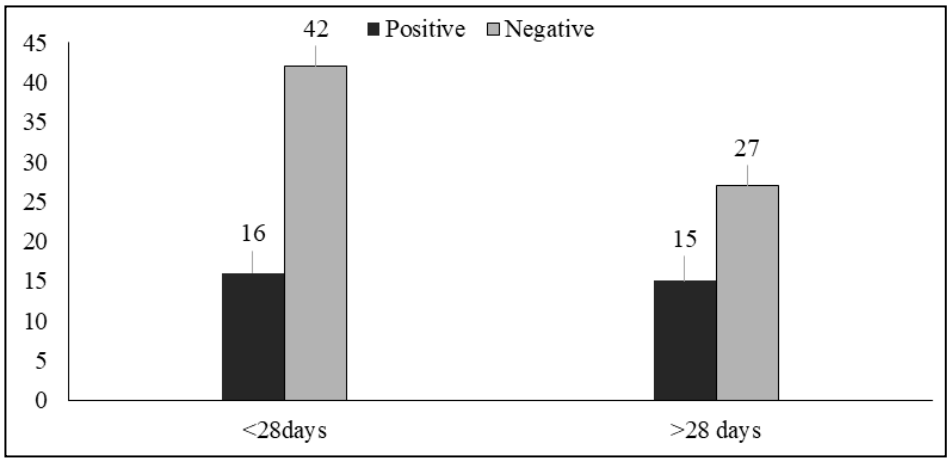

Fig 2: Bar graph showing the prevalence of E. coli in Surkhet district according to age

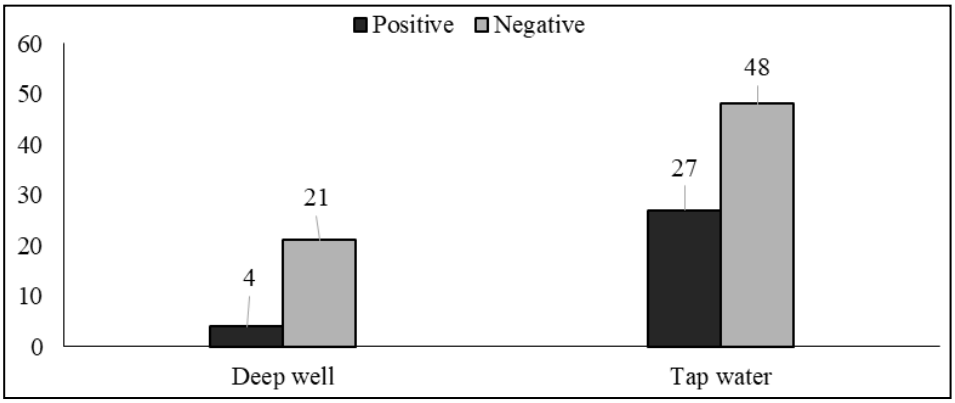

Fig 1: Bar graph showing the prevalence of *E. coli* in Surkhet district according to source of water

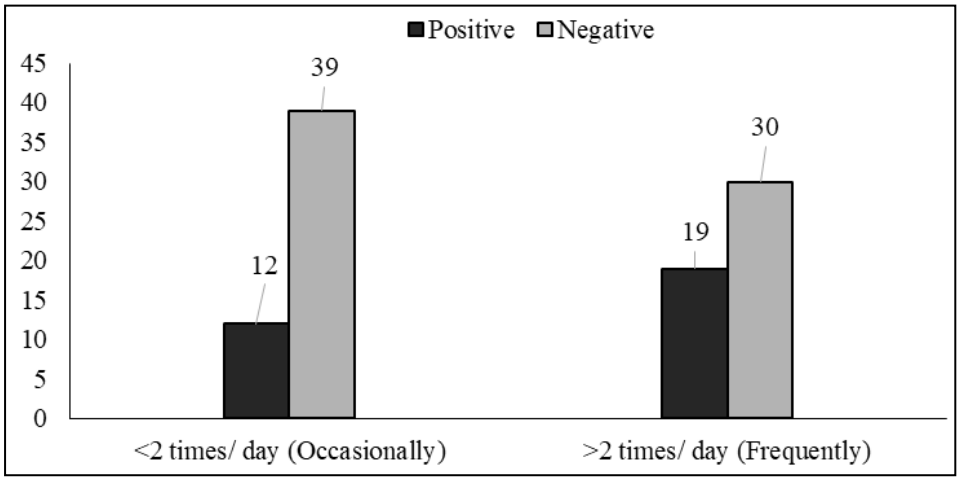

Fig 2: Bar Graph Showing the Prevalence of *E. coli* in Surkhet district according to frequency of cleaning drinker

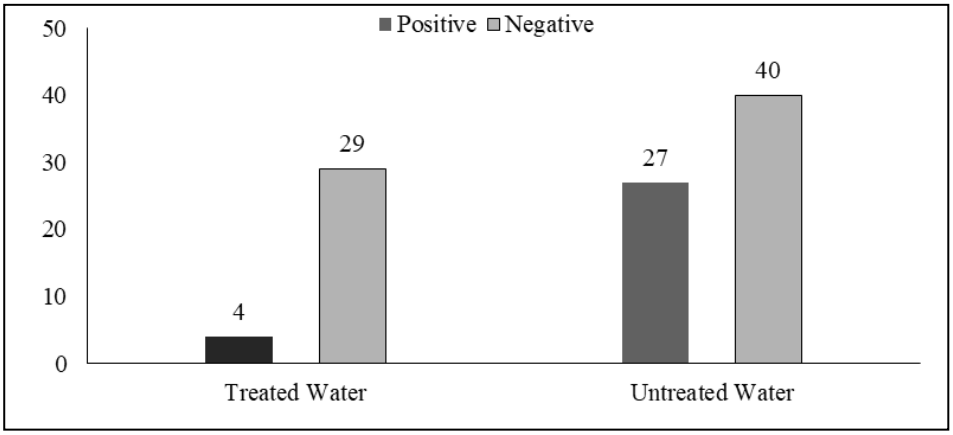

Fig 3: Bar Graph showing the prevalence of *E. coli* in Surkhet district according to Status of water used

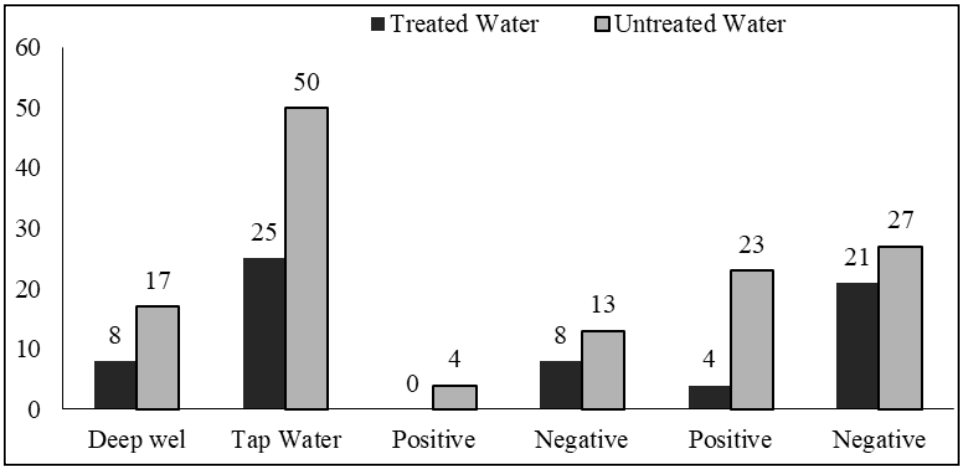

Fig 4: Bar graph showing the prevalence of *E. coli* in Surkhet district according to status of water used and sources of water used accordingly

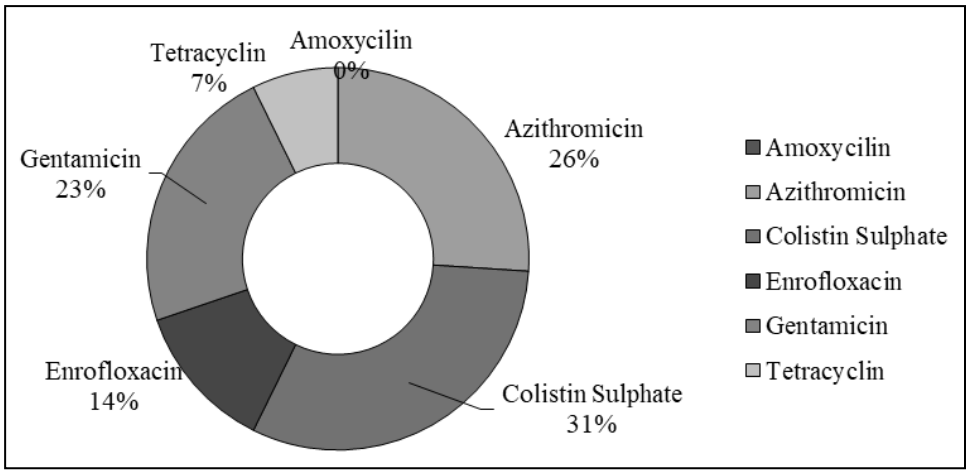

Fig 5: Showing antibiotic sensitivity pattern of different antibiotics

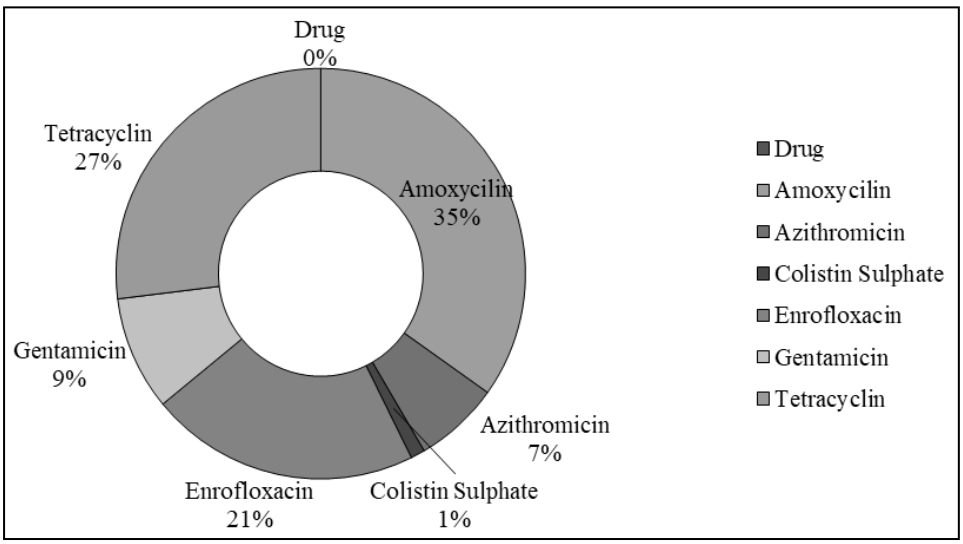

Fig 8: Showing resistivity pattern of different antibiotics

Discussion

The overall prevalence of *E coli was* found to be 31%. On statistical analysis for the risk factors according to age, source of water and frequency of clean drinker, the result was found to be non- significant ($P>0.05$) and status of water (treated/untreated)was found to be significant ($P<0.05$). The antibiotic sensitivity test on the positive case were found to be: Colistin sulphate-96.77%, Azithromicin,-80.64%, Gentamicin-70.96%, Enrofloxicin-38.71%, Tetracyclin-22.58%, Amoxycilin-0% sensitive and Amoxycilin-100%, Tetracyclin-77.42%, Enrofloxicin-61.29%, Gentamicin-25.80%, Azithromicin-19.35%, Colistin sulphate-3.22% resistant respectively. Out of total 100 cases, 75 poultry farmers used tap water and 25 used deep well as a source of water. Out of the 31 positive cases from Surkhet, 4 were obtained from deep well and 27 were obtained from tap water source for drinking purpose in poultry. All the positive cases using deep well as water source, were not treated by water sanitizer. But, in positive cases using tap water, 4 were found undergoing water treatment while the rest of 23 didn't used any water sanitizer.

Acharya (2011) reported that prevalence rate was 13.33% from the sample of western Chitwan, which is lower than my present findings. Difference might be due to geographical variation.

Maharjan (2011) reported to 41.63% overall prevalence in the study of "Relationship of *E .Coli* Infection in Poultry with Respect to Its Prevalence in Drinking Water Sources Used in Poultry Farms of Kathmandu Valley" which is higher than my findings. Difference in the findings might be due to difference in location, season and degree of pollution.

Hossain (2006) reported 63.5% prevalence of *E. coli* in healthy broilers from farms near Bangladesh Agriculture University which is quite higher than my findings. Difference in the findings might be due to the reason that the sample size was larger in his research and more over it was done in the colibacillosis outbreak period.

Shrestha *et al.* (2008) reported 28% prevalence of *E. coli* in the study of "Antimicrobial Resistance Pattern of *E.coli* Isolates from Chicken and Human Samples in Chitwan" which is slightly lower than my finding. Difference in findings might be due to the difference in sampling procedure, season and location Sapkota *et al.* (2006) [20] reported that 12.22 % prevalence of *E .coli* in cases of poultry brought for PM examination at VTH, Rampur, Chitwan which is much lower than my findings. Difference in findings might be due to the difference in sampling and test procedure as well as geographical variation.

In one study conducted in NAL, Bharatpur reported that 13.97% prevalence of *E. coli* in Chitwan valley (Neupane, *et al.*, 2005) [16] which is much lower than my findings which may be due to spatial difference and time of research.

Dhakal *et al.* (2000), reported that the prevalence of *E. coli* in Padampokhari village of Makawanpur district was 11%, which is much lower than my finding which may be due to the geographical variation.

Dhakal (2002) [8] reported 18.20% prevalence rate in the year 1999 in Chitwan which is lower than my finding which may be due to the fact that the research was carried out considerably earlier in time and the progress of disease agent may not be too much.

Pandey (2003) [17, 18] reported about 6.98% cases of *E. coli* infection in Chitwan district which is very much lower than my finding. It may be due to geographical variations.

Overall prevalence of colibacillosis was recorded as 77.78% by Islam (2014) in Bangladesh which is quite higher than my findings. Difference in the findings might be due to the reason that the sample size was larger in his research and more over it was done in the colibacillosis outbreak period.

Olarinmoye *et al.* (2013) found that *E. coli* were highly resistant to amoxicillin (99.2%), tetracycline (91.4%); moderately resistant to gentamicin (67.2%) which is quiet similar with my study in relation to amoxicillin (100%) and less as tetracycline (77.42%) with great variation on gentamycin (25.80%) might be due to the different testing procedure.

5. Conclusions

In this research the overall prevalence of *E. coli* was found to be 31%. The risk factors that were considered as risk for the bacterial infestation were found to be statistically non-significant but for the status of the water (treated/untreated) it was found to be statistically significant. Thus, farmers are recommended to use the treated water in order to decrease the chance of *E. coli* infestation.

Further, proper hygiene should be maintained in and around the farms and the poultry waste and excreta should be disposed properly. Overcrowding and stress are to be avoided as far as possible. Use of antibiotic should be made only after proper and regular sensitivity analysis.

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