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## Prevalence of Colibacillosis in Young Broiler Chicks and Antibiogram of *Escherichia coli* in Different Areas of Hazara Region

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## Abstract

**Background:** Poultry sector is a vital zone of Pakistan economy and is a feasible source of animal derivative protein. In term of mortality and morbidity the development of this segment is significantly affected by a lot of problems. Hazara region is well known for poultry in Pakistan. Colibacillosis, caused by *Escherichia coli* is vital disease among poultry of all ages resulting in huge economic losses. The aim of this study was to evaluate the prevalence of colibacillosis in young broiler chickens and antibiogram of *E. coli* in different areas of Hazara region.

**Methods:** The current study was conducted to evaluate the prevalence of colibacillosis in young broiler chickens and antibiogram of *E. coli*. This study was done during February 2019 to June 2019; a total of 200 (n=200) liver samples of freshly dead young broiler chicks were randomly collected from the postmortem section of Veterinary research and disease investigation center Abbottabad (VRDICA). The identification of *E. coli* was confirmed by gram staining and biochemical tests. Susceptibility pattern to 13 antibiotics was also checked.

**Results:** Total of 200 (n=200) samples were brought to the VRDICA for the diagnosis of disease and antibiotic susceptibility testing. Out of these 130 were positive (Overall prevalence =65%). Area wise prevalence varied from area to area and highest prevalence was recorded in Abbottabad (81.11%) followed by Havelian (75%), Manghal (72.22%), Mansehra (60%), Qalandarabad (50%) and Haripur (28.57%). About age group, highest mortality was found in 11-15 days old chicks (93%) as compared to 6-10days (83.33%) and 1-5 days old chicks (21.42%). Antibiogram showed highest sensitivity to Gentamicin (100%) afterward Colistin Sulphate (92.30%). However, lincomycin (92%) and streptomycin (96%) display highest resistance.

**Conclusion:** In this study, the highest prevalence of infection was observed in young chicks that play a crucial role in their mortality. *E. coli* had showed resistance to commonly recommended antibiotic so care should be taken while prescribing the drugs.



## Introduction

Colibacillosis is a major widespread infection of fowl and it is well thought out to increase the morbidity and mortality in immature (young) chicks as compare to the older ones [1]. Majority of this disease is established among chickens, which are two to ten weeks old [2]. During Colibacillosis plentiful lesions are observed on different organs, usually peritonitis, airsacculitis, perihepatitis, pericarditis, while additional lesions are extra-intestinal [3, 4]. Enterobacteriaceae family contain gram negative bacteria and some of its members chiefly infect broilers in different stages of their life and *Escherichia coli* is the most prevalent member among them. Antibiotics are regularly cast-off as therapeutic agents in poultry production and could lead to the development of antimicrobial resistance [5] and bacteria that are diversely opposed to medication carried are competent to infect humans by intervention in foodstuff all the way by utilization of bird meat or any other product of poultry [4,6].

This research anticipated for the isolation and identification of *E. coli* from the liver of freshly dead young broiler chicks for the definitive diagnosis of suspected disease (Colibacillosis), to find out the prevalence of Colibacillosis affected chicks on account of age and area along with verification of Antibiogram summary of *E. coli* to 13 commonly used antibiotics.

## Methods

This study was carried out in VRDICA from Feb to June 2019. During postmortem of freshly dead young broiler chicks, in some cases, characteristic necropsy findings of Colibacillosis viz; airsacculitis, perihepatitis, pericarditis and whitish layer on all visceral organs especially liver and heart were observed. After necropsy, 200 liver samples were randomly assembled in sterile plastic bags and were shifted to Microbiology lab of the center in order to isolate, identify the *E. coli* for the definitive diagnosis of colibacillosis.

A loopful from the liver samples was directly streaked on MacConkey agar plates and incubated at 37°C for 24 hours for the initial screening of bacteria. After incubation, the specific colony was sub-cultured on Eosin Methylene Blue Agar (EMB) agar, and then incubated for overnight at 37°C. The suspected *E. coli* colonies were subjected to characterization by studying their morphological, cultural and biochemical characteristics. Gram staining and IMVIC test were performed for identification or confirmation of *E. coli*. Then *E. coli* were inoculated on Muller Hinton agar. Thirteen (13) different antibacterial discs were fixed superficially on the lawn culture, incubated at 37°C for 24 hours.

Different antibacterial discs were used such as amoxicillin (10µg/disc), doxycycline (30µg/disc), enrofloxacin (5µg/disc), colistin sulphate (10µg/disc), erythromycin (15 µg /disc), gentamicin (10 µg/disc), oxytetracycline (30 µg/disc), penicillin G (10µg/disc), tylosin (30 µg/disc), tilmicosin (15µg/disc), trimethoprim (5µg/disc), lincomycin (10µg /disc) and streptomycin (10µg/disc) by Kirby-Bauer method [7]. The antibiotic

susceptibility pattern of the isolates was interpreted according to the standard followed in VRDICA.

## Results

The results of the bacteriological studies of 200 liver samples of chicks revealed 130 (65%) over all prevalence of Colibacillosis caused by *E. coli* as presented in **Table 1**. Age wise prevalence is stated in **Table 2**, acclaimed that it was greater in 11-15 days old chickens which was about 92.85% while 83.33% was noted in 6-10 and 21.42% in 1-5 days old chicks respectively. The Area wise Prevalence of Colibacillosis affected chicks is demonstrated in **Table 3**, showed that the highest prevalence was observed in Abbottabad (81.11%), followed by (75.00%), (60.00%) and (28.57%) in Havelian, Mansehra, and Haripur respectively.

### Antibiogram Pattern of *E. coli* by Disc Diffusion Method

In the present study, Antibigram profile of *E. coli* has been shown in **Table 4**. Expressively sensitive to Gentamicin completely followed by Colistin sulphate (92.2%) and Tilmicosin (65.38%). However, it was highly resistant to streptomycin (96.15%) followed by lincomycin (92.30%) and doxycycline (69.23%) correspondingly.

Chicken Line	Liver samples observed	Colibacillosis positive cases	Overall prevalence %
Freshly dead young broiler	200	130	65%

**Table 1:** Overall prevalence of colibacillosis affected chicks. Over all 200 liver samples of freshly dead young broiler chicks were examined. When growth characteristics were observed, it was then confirmed that 130 chicks were affected by *E. coli*. The overall prevalence of Colibacillosis affected chicks was 65%, which is an alarming situation regarding the health of young chicks.

Area	Liver samples observed	Colibacillosis positive cases	Prevalence %
Abbottabad	90	73	81.11
Manghal	18	13	72.22
Havelian	12	9	75.00
Qalandarabad	20	10	50.00
Mansehra	25	15	60.00
Haripur	35	10	28.57
Total	200	130	65.00

**Table 2:** Area-wise prevalence of colibacillosis affected chicks. The highest prevalence rate of colibacillosis affected young chicks was observed as 81% in Abbottabad area. In contrast to Abbottabad region, the least affected young chicks were noticed in Haripur region with the prevalence percentage of 28.57 (29%) which is very low as compared to other areas.

Age groups	Total liver Samples observed	Colibacillosis positive cases	Prevalence %
1-5 days	70	15	21.42
6-10 days	60	50	83.33
11-15 days	70	65	92.85
Total	200	130	65.00

**Table 3:** Age-wise prevalence of colibacillosis affected chicks. According to this, *E. coli* extensively affect the chicks in 11-15 days old age and these chicks have a high mortality rate 92.85 (93%) as compared to other age groups.

## Discussion

Our study recorded 65% prevalence of colibacillosis in broiler and it is supported by the findings of Srinivasan et

Antimicrobial agents	Code	Conc per disc in µg	Total <i>E. coli</i> isolates	Antibiogram profile of <i>E. coli</i>		
				Susceptible (S) %	Intermediate (I) %	Resistant (R) %
Amoxicillin	AML	10	130	0(0)	20(15.4)	110(85)
Doxycycline	DO	30		0(0)	40(30.8)	90(69.23)
Enrofloxacin	ENR	5		0(0)	65(50)	65(50)
Erythromycin	E	15		15(11.53)	80(61.5)	35(27)
Colistin sulphate	CS	10		120(92.30)	10(7.7)	0(0)
Gentamicin	CN	10		130(100)	0(0)	0(0)
Oxytetracycline	OT	30		0(0)	80(62)	50(38.46)
Penicillin-G	P	10		0(0)	58(44.61)	72(55.38)
Tylosin	TY	30		0(0)	70(53.84)	60(46.15)
Tilmicosin	TIL	15		85(65.38)	45(34.61)	0(0)
Trimethoprim	W	5		20(15.38)	110(84.61)	0(0)
Lincomycin	MY	10		0(0)	10(7.7)	120(92.30)
Streptomycin	S	10		0(0)	5(3.8)	125(96.15)

**Table 4:** Results of Antibiogram profile of *E. coli*. Few of the *E. coli* isolates revealed complete sensitivity to some antibiotics while most of the isolates exposed resistance and intermediate sensitivity to the most commonly used antibiotics. The data related with the susceptibility pattern of *E. coli* clearly demonstrated that Gentamicin and Colistin sulphate may be used for the treatment of those young broiler chicks which could be affected with colibacillosis.

*al.*, [8] which stated that higher prevalence of colibacillosis caused by *E. coli* was found in broiler chicken (87.5%). The age wise prevalence of colibacillosis affected chicks in the present study was in agreement with the findings of [2] who reported the most common occurrence of this disease among 2 to 10 week-old chicken. However, the age wise mortality rate due to this disease in young broiler chicks (recorded in this study) may be due to their suppressed immune system.

In this study young broiler chicks were fetched from different geographical areas located in Hazara division and it was found that those broiler chicks were extremely affected with colibacillosis 73(81.11%) in Abbottabad. However it was also distinguished that prevalence of this disease varied greatly area wise. This was in accord with findings of Biswas *et al.*, and Tonu *et al.*, [9, 10] which recorded that prevalence of colibacillosis was affirmed as 28% in Sonali chicken in Bangladesh (on smallholder households) and 32.6% in flock of broiler in South Korea respectively. Hence, Area wise variation in prevalence of colibacillosis may be related to dust in poultry houses, food hygienic conditions and hatchery problems especially fecally contaminated eggs. The antibiogram pattern of *E. coli* revealed that isolates were sensitive to Colistin sulphate. Similar results were also reported by [11] while our results illustrated divergence with the findings of [12-14] where isolates of *E. coli* showed 25% sensitivity to Gentamicin while in present study *E. coli* isolates showed high level of sensitivity to Gentamicin (100%). Table 4 proclaim that isolates of *E. coli* showed highest sensitivity to Gentamicin (CN 10µg/disc) and Colistin sulphate (CS 10µg/disc). These results also agreed with findings of [14] who reported that *E. coli* isolates were sensitive to Gentamicin and Colistin sulphate. Now it is quite clear from Table 4, that many of *E. coli* isolates showed resistivity while few of them showed intermediate sensitivity to most common antibiotics. So it is strongly recommended to decrease the use of unethical antibiotics to minimize the development of resistant strain.

**Ayesha Tahir:** Primary and main author, organized and conducted the experimental work, data mining and write article.

**Kayanat Bibi:** Conducted the laboratory work, and write article.

**Sana Waheed:** Conducted the laboratory work.

**Muhammad Azam Khan:** Reviewed and Supervised.

**Farwa Ayaz & Fatma Rauf:** Rewrote and reviewed article.

## Competing Interest

All authors declare no conflicts of interest in this paper.

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## Author Contributions

