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**Research Article**

# Survey of antibiotic treatment of *Escherichia coli* infection in broilers and efficacy of Enrofloxacin plus colistin in experimental Colibacillosis

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**Abstract**

Colibacillosis caused by *Escherichia coli* is very important disease of poultry and is characterized by development of colisepticemia, pericarditis, perihepatitis, increased mortality and decreased feed conversion rate. Thirty *E. coli* infected broiler farms located in district Faisalabad were surveyed and data pertaining to antibiotic usage, storage, withdrawal period and farmers perception on antibiotic resistance was obtained. Data showed that most of poultry farmers used antibiotics in combination (Enrofloxacin plus colistin) to treat or control *E. coli* infection. Morbid samples from suspected *E. coli* infected birds were collected, pure colonies of were isolated and pathogenicity was determined. *In vivo* colibacillosis in broilers was induced by oral inoculation of $1\times10^6$colony forming units (cfu)/bird and efficacy of enrofloxacin plus colistin was determined. For that purpose one hundred forty (140) broiler birds were divided into four groups (A, B, C and D), each having 35 birds. At 26$^{th}$ day of age *E. coli* infection was induced in all groups. At 28$^{th}$ day of age group A was treated with enrofloxacin plus colistin sulphate, group B with enrofloxacin, group C with colistin sulphate and group D was kept as untreated control. Mortality, cumulative feed consumption, mean body weight, mean weight gain and FCR were recorded in each group. It was found that combined use of enrofloxacin plus colistin in colibacillosis in broilers decreased mortality, increased cumulative feed consumption, increased mean body weight, increased mean weight gain and FCR than single use of either of enrofloxacin or colistin.

**Keywords:** Antimicrobial therapy; Colibacillosis; Poultry flocks

## Introduction

Colibacillosis caused by *E. coli* is a disease of significant economic concern in all phases of poultry industry in Pakistan. *E. coli* are worldwide in distribution and are found in intestinal tract of all warm blooded species, also found in the litter and fecal material and persist for long period of time

under farm conditions. Contaminated feed and drinking water act as a source of colibacillosis. Egg transmission of pathogenic *E. coli* cause high early chick mortality and morbidity. Prevalence rate of colibacillosis in poultry is 13.44% [1]. Higher incidence of colibacillosis in poultry is also reported [2-5].

A number of antibiotics are used to treat and prevent bacterial diseases of poultry. Strains of *E. coli* are generally resistant to a variety of antibiotics used as therapeutic, preventive or growth promoting agents [6-8]. No single antimicrobial agent may be effective against all *E. coli* isolates in a geographic region [5]. As a result there is a need for conjoined antimicrobial therapy.

Enrofloxacin is carboxylic acid quinolone derivative, used for combating many bacterial diseases. It has a wide antibacterial spectrum against mycoplasma, salmonella, *E. coli*, *Haemophihus paragalinarum, Pasteurella multocida* and staphylococcus in chicken [9-11]. Colistin (polymyxin E) is a cationic surface-active agent that disrupts the structure of cell membrane phospholipids and increases cell permeability by a detergent like action. It disorganizes the outer membrane of bacteria by binding to phospholipid (endotoxin) through direct interaction of the cationic drugs with the anionic lipid-A region. Colistin is synergistic with a variety of antimicrobial drugs in its disorganizing effects on the outer and cytoplasmic membrane. It is used in veterinary practice against many bacterial diseases in chicken and is effective against gram-negative bacteria such as *E. coli*, salmonella, pasteurella and pseudomonas [12]. Although this antibiotic has been commonly prescribed in combination with enrofloxacin. The published report on the efficacy of this combination under Pakistani conditions is lacking. The present study was therefore, designed to investigate the therapeutic efficacy of combination of enrofloxacin and colistin in an experimentally induced colibacillosis in broiler chicks.

## Materials and methods

### Part - I

### Field Survey of antibiotic usage for treatment of *E. coli*

Thirty *E. coli* infected poultry farms located in district Faisalabad were surveyed and following data was obtained.

1. Antibiotic usage (single or combination, name of antibiotic, brand name, manufacturer), pre-treatment, post treatment mortality and percent reduction of mortality.
2. Awareness of antibiotic usage patterns i.e. duration, efficacy, storage, price, brand, with drawl period, etc.

### Part - II

### Isolation and identification of *E. coli*

Livers and hearts of sick and dead birds of different age groups suffering from suspected *E. coli* infection were collected. Samples were transported to the laboratory and inoculated on MacConkey's agar medium for primary isolation, purification and identification of *E. coli* from samples [13].

### Morphology and staining reaction

The morphological, cultural, staining characteristics, of isolates were determined. The smears were prepared and stained with gram's staining and examined under microscope. The morphology and staining reaction of each isolate was recorded. For biochemical and pathgenicity test, the isolates were maintained on MacConkey's agar slants.

### Sugar fermentation and Biochemical tests

Sugar fermentation and biochemical tests including triple sugar iron reaction and citrate utilization test were performed for confirmation of *E. coli* [13-15].

### Pathogenicity of *E. coli*

Gut loop assay was performed for determination of pathogenicity of *E. coli*. The rabbits were anesthetized with Ketamine HCI and the ileum was exposed. The ileum was cut and half milliliter culture of the test isolate was delivered into the lumen of the intestine. Equal volume of normal saline was injected into the intestine

in control group. The lumen of intestine was sutured with catgut and animals were kept under care. The observations were recorded [16].

**Preparation of inoculum**

After confirmation of the pathogenicity, *E. coli* isolate was inoculated into fresh nutrient broth and incubated at 37ºC for overnight. The viable count of bacteria was carried [13]. The broth was diluted with phosphate buffer saline (PBS) to have approximately $1\times10^6$cfu/mL. The inoculums were further used for experimental infection.

**Experimental design**

140 day old broiler chicks (Hubard × Hubard) were purchased and were reared under uniform conditions of management and nutrition. The birds were fed with broiler starter ration from day 1 to 28$^{th}$ days and broiler finisher ration from 29$^{th}$ to 40$^{th}$ days. All the birds were vaccinated according to the schedule (Table 1).

At 25$^{th}$ day, birds were divided into four groups (A, B, C and D), each having 35 birds. At age of 26$^{th}$ day *E. coli* infection was induced by oral inoculation of 1mL of $1\times10^6$ cfu/bird in all groups. At 28$^{th}$ day of age Group A was treated with Enrofloxacin plus Colistin Sulphate, group B with Enrofloxacin, group C with Colistin Sulphate and group D was kept as untreated control (Table 2).

Mortality, postmortem findings, cumulative feed consumption, weight gain and feed conversion ratio and in each group was recorded and results were compared. Data was statistically analyzed / compared accordingly.

**Results**

Data collected from thirty *E. coli* infected farms showed that antibiotics in *E. coli* infection were mostly used in combination. Most frequently used combination contained enrofloxacin plus colistin (Table 3). Data also revealed that most of the farmers were totally illiterate about antibiotic usage, efficacy, storage and recommended dosage of different antibiotics (Table 4).

**Effect of treatment on mortality in groups**

Observations with respect to mortality are shown (Table 5). Total mortality throughout the entire experimental in all groups was 30.

**Postmortem findings in groups**

Thickened and cloudy air sacs and in severe cases, caseous exudates on air sac was present. Adhesive pericarditis and fibrinous perihepatitis were present in most of the cases. Intestinal infections with enteritis with excessive mucus and diarrhea (yellowish droppings) were common.

**Effect of treatment on cumulative feed consumptions, weight gain and FCR in groups**

Effect of treatment on cumulative feed consumptions, weight gain and FCR in groups has been shown (Table 6-9).

**Discussion**

To evaluate the efficacy of conjoined therapy (enrofloxacin plus colistin) under *in vivo* condition, pathogenic strains of *E. coli* were selected. For identification of *E. coli*, a smear was prepared from growth on MacConkey's agar and stained with gram-staining. The isolates were cocco-bacillary, long filamentous and pinkish in color. These cultural and morphological characteristics are consistent with previous reports [17].

**Table 1. Vaccine schedule of experimental chicks**

| Age(Days) | Name of Vaccine | Shot (1$^{st}$ / 2$^{nd}$) | Route |
|---|---|---|---|
| 5 | Newcastle disease (ND) | 1$^{st}$ | E/D |
| 10 | Infectious bursal disease (IBD) / Gumboro | 1$^{st}$ | E/D |
| 17 | Hydropericardium syndrome | Single | S/C |
| 20 | IBD | 2$^{nd}$ | D/W |
| 24 | ND | 2$^{nd}$ | D/W |

E/D = Eye drop, S/C = Sub Cutaneous, D/W = Drinking water

**Table 2.Antibiotic treatments in different groups**

| Group | A | | B | C | D |
|---|---|---|---|---|---|
| **No. of birds** | 35 | | 35 | 35 | 35 |
| **Quantity of inoculums** | 1mL | | 1mL | 1Ml | 1mL |
| **Concentration of inoculums** | $1\times10^6$ cfu | | $1\times10^6$ cfu | $1\times10^6$ cfu | $1\times10^6$ cfu |
| **Antibiotic usage (combination / single)** | Combination of two antibiotics | | Single | Single | Nil |
| **Treatment (antibiotic usage)** | Enrofloxacin | Colistin sulphate | Enrofloxacin | Colistin sulphate | Nil |
| **Dose rate (@)** | 1mL / 4L | 1gm / 10L | 1mL / 4L | 1gm / 10L | Nil |
| **Route of treatment** | DW | DW | DW | DW | Nil |
| **Name of brand** | Vet Enrox-20 | Colisol | Vet Enrox-20 | Colisol | Nil |
| **Name of manufacturer** | Vetycare Pharma, Pak | Vetycare Pharma, Pak | Vetycare Pharma, Pak | Vetycare Pharma, Pak | - |
| **Duration of treatment (days)** | 5 | | 5 | 5 | - |

**Table 3.Field survey of antibiotic usage in *E. coli* associated problems on 30 farms in Faisalabad district.**

| No. of Birds | Mortality | | | Brand name | Name of manufacturer | Antibiotic usage (single / combination) |
|---|---|---|---|---|---|---|
| | Pre-treatment | Post-treatment | Reduction (%) | | | |
| 2000 | 50 | 15 | 2.2 | Aitquine 20% | Azieda Therapeutica, Italy | Single |
| 4000 | 250 | 20 | 5.75 | Enrosol-S | Vet. & Agri., Product, Jordan | Combination |
| | | | | Colisol | Do Pharma Netherlands | |
| 3000 | 120 | 18 | 3.34 | Vetyenrox 10% | Vetycare Pharma, Pak | -do- |
| | | | | Colimysin | Gellini Intl., Italy | |
| 10,000 | 250 | 52 | 2 | Floxamycin | Vaksindo Pharma, Indonesia | Single |
| 3000 | 189 | 50 | 4.63 | Vetyerox 10% | Vetycare Pharma, Pak | Combination |
| | | | | Tribressin | Glaxo Welcome, Pak | |
| 3000 | 140 | 25 | 3.83 | Vetyenrox-20% | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 4000 | 398 | 85 | 7.85 | Enrosol-S | Vet. & and Agri. Products, Jordan | -do- |
| | | | | Colimycin | Gellini Intl., Italy | |
| 2500 | 200 | 120 | 3.2 | Vetyerox10% | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 3500 | 450 | 125 | 11.28 | Vetyenorx20 % | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 2200 | 290 | 108 | 8.27 | Vetyenrox20% | Vety-care Pharma, Pak | -do- |
| | | | | Colimycin | Gellini Intl., Italy | |
| 2700 | 185 | 130 | 2.03 | Aitquine | Zieda Therapentica, Italy | Single |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3200 | 300 | 65 | 7.34 | Enrosol-S | Vet.& Agri. Products, Jordan | Combination |
| | | | | Colimycin | Gellini Intl., Italy | |
| 4000 | 311 | 105 | 5.15 | Enrosol-s | Vet. & Agri. products, Jordan | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 3800 | 296 | 90 | 5.43 | Vetyenrox 10% | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 3200 | 320 | 100 | 6.87 | Enrosol-S | Vet. & Agri. products, Jordan | -do- |
| | | | | Colisol | Do Pharma, Netherlands | |
| 2500 | 150 | 69 | 3.24 | Floxamycin | Vaksindo Pharma Indonesia | Single |
| 3500 | 320 | 100 | 6.28 | Vetyenrox 10% | Vetycare Pharma, Pak | Combination |
| | | | | Colisol | Do Pharma Netherlands | |
| 4200 | 435 | 80 | 8.45 | Enrosol-S | Vet. & Agri. products, Jordan | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 2000 | 158 | 80 | 3.25 | Flumiquine-20% | -- | Single |
| 7500 | 492 | 90 | 5.36 | Enrosol-S | Vet. & Agri. products, Jordan | Combination |
| | | | | Colisol | Do Pharma Netherlands | |
| 2500 | 205 | 180 | 5.46 | Vetyenrox 10% | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 5500 | 450 | 125 | 5.90 | Vetyenrox20% | Vetyare Pharma, Pak | -do- |
| | | | | Colimycin | Gellini Intl., Italy | |
| 3100 | 220 | 105 | 3.7 | Aitquine | Zieda Therapentica, Italy | Single |
| 2800 | 390 | 127 | 9.39 | Tribressin | Vet. & Agri. products, Jordan | Combination |
| | | | | Enrosol-S | -do- | |
| 6000 | 440 | 135 | 5.08 | Vetyerox-10% | Vetycare Pharma, Pak | Single |
| 5000 | 305 | 90 | 4.43 | Aitquine | Zieda Therapentica, Italy | -do- |
| 2900 | 297 | 85 | 7.41 | Vetyenrox 10% | Vetycare Pharma, Pak | Combination |
| | | | | Colimysin | Gellini Intl., Italy | |
| 2500 | 400 | 290 | 4.4 | Vetyenrox 10% | Vetycare Pharma, Pak | -do- |
| | | | | Colisol | Do Pharma Netherlands | |
| 2800 | 510 | 235 | 9.82 | Atiquine 20 | Azieda Therapcutica Italy | -do- |
| | | | | Colisol | Do Pharma Netherlands | |

**Table 4. Awareness of farmers to antibiotic usage patterns**

| Parameters | | Range of Poultry Farms | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 |
| Source of Antibiotic | Medical store | | √ | | | | | |
| | Any other source | | | √ | | | | |
| | Distributer | | | | √ | | | |
| Antibiotic Prescriber | Veterinarian | | | | √ | | | |
| | Distributor | | | √ | | | | |
| | Self | | √ | | | | | |
| Antibiotic dosage | | | | | √ | | | |
| Antibiotic withdrawal period | | √ | | | | | | |
| Price and antibiotic selection | | | | | | | | √ |
| Proper storage at farm* | | √ | | | | | | |
| Antibiotic ineffectiveness** | | | | | | | | √ |
| Resistance to antibiotics | | | | | | | | √ |
| Irrational use of antibiotics | | | | √ | | | | |
| Support for combing antibiotics | | | | | | √ | | |

*Proper storage in the context of this survey means storage in a room without direct sunshine, inside temperature not exceeding 30$^{o}$C and is rodent proof.

**Perceptions concerning the reduction of effectiveness of antibiotics used at least during the past 5 years.

**Table 5. Mortality in experimental groups**

| Age (Days) | DPI | Mortality in experimental groups | | | | Total Mortality |
|---|---|---|---|---|---|---|
| | | A | B | C | D | |
| 27$^{th}$ | 1$^{st}$ | - | - | - | - | - |
| 28$^{th}$ | 2$^{nd}$ | 1 | 4 | 3 | 5 | 13 |
| 29$^{th}$ | 3$^{rd}$ | 1 | 3 | 2 | 3 | 9 |
| 30$^{th}$ | 4$^{th}$ | - | 1 | 1 | 2 | 4 |
| 31$^{st}$ | 5$^{th}$ | - | - | - | 2 | 2 |
| 32$^{nd}$ | 6$^{th}$ | - | - | - | - | - |
| 33$^{rd}$ | 7$^{th}$ | - | - | - | 2 | 2 |
| 34$^{th}$ | 8$^{th}$ | - | - | - | - | - |
| 35$^{th}$ | 9$^{th}$ | - | - | - | - | - |
| 36$^{th}$ | 10$^{th}$ | - | - | - | - | - |
| 37$^{th}$ | 11$^{th}$ | - | - | - | - | - |
| 38$^{th}$ | 12$^{th}$ | - | - | - | - | - |
| 39$^{th}$ | 13$^{th}$ | - | - | - | - | - |
| 40$^{th}$ | 14$^{th}$ | - | - | - | - | - |
| Total = | | 2 | 8 | 6 | 14 | 30 |
| Percentage (%) = | | 5.71 | 22.85 | 17.14 | 40 | 21.42 |

**Table 6. Cumulative feed consumption (Kg) igroups**

| Age (Days) | Cumulative feed consumption (Kg) in groups | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 25$^{th}$ | 35.6 | 35.6 | 35 | 34.2 |
| 30$^{th}$ | 70.20 | 68.2 | 66.9 | 56.7 |
| 35$^{th}$ | 101.38 | 80.5 | 86.37 | 60.4 |
| 40$^{th}$ | 140.7 | 95.30 | 112.23 | 64.44 |

**Table 7. Mean body weight in groups**

| Age (Days) | Mean body weight (gm) in groups | | | | Mean |
|---|---|---|---|---|---|
| | A | B | C | D | |
| 25th | 436.51 | 422.85 | 416.54 | 434.4 | 427.579 |
| 30th | 800.81 | 724.39 | 726 | 565.28 | 590.157 |
| 35th | 1200.06 | 1125.40 | 1099.79 | 863 | 863..43 |
| 40th | 1645.09 | 1406.90 | 1456 | 1092.61 | 1092.4 |
| Mean | 956.786 | 728.921 | 778.721 | 509.058 | -- |

**Table 8. Mean weight gain in groups**

| Age (Days) | Mean weight gain (gm) in groups | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 25th | - | - | - | - |
| 30th | 11.03 | 8.61 | 10.31 | 5.23 |
| 35th | 12.9 | 12.32 | 12.88 | 13.53 |
| 40th | 13.90 | 10.42 | 12.72 | 10.93 |

**Table 9. Feed conversion ratio in groups**

| Age (Days) | FCR in groups | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 25th | 2.26 | 2.33 | 2.33 | 2.18 |
| 30th | 2.49 | 2.77 | 2.93 | 2.72 |
| 35th | 2.48 | 2.55 | 2.61 | 2.79 |
| 40th | 2.45 | 2.51 | 2.65 | 2.89 |

Biochemical reactions of *E. coli* isolates were studied. On triple sugar iron agar, the isolates fermented lactose and sucrose with production of gas, but hydrogen sulphide was not produced on Simon Citrate medium, the isolates change the color of the medium from original green to blue and colonies were streaked on the medium. Similarly, researchers studied the biochemical reaction of 267 strains of *E. coli* and found fermentation of glucose with the production of acid and gas, sucrose was fermented by 153 strains manitol by 267, salicin by 14 and dulcitol by 161 with20 late fermenters. Methyl red was positive for 267. However, none of strains produced hydrogen sulphide **[18]**. All *E. coli* isolates are not pathogenic and is quite necessary to distinguish between pathogenic and non-pathogenic strains.

In our study the pathogenicity of *E. coli* was determined using intestinal loope ligation method. Researchers had also adopted intestinal loop ligation method for the evaluation of pathogenicity of *E. coli* **[19]**. In the present study intestinal loop ligation method, the positive samples (i.e pathogenic) of *E. coli* were identified by increased size (i.e triple fold than normal) and increased luminal fluid of the intestine. Present results also coincide with the previous studies **[16]**.

*In vivo*, the disease was produced experimentally by oral administration of $1\times10^6$ cfu (bacilli) / mL in all the groups at the age of 26th days. The experimental disease of *E. coli* can also be produced through other routes i.e. intravenous, etc as described by different method **[20, 21]**.

In the present study primary lesions of experimentally induced colisepticemia were pericarditis, perihepatitis and air sacculitis. Pericardial fluid became progressively more fibrinous. Necrotic foci in the heart muscles were present in the chicks which succumbed to infection. Pericardial sac was thickened and pale colored gelatinous exudates. Congestion of liver, spleen, kidney and small intestine were also observed. Similar lesions are also

reported [22-24]. The present findings of described pathological lesions also coincide researchers who found petechiation of intestines, heart, lungs, kidneys and enlargement of liver in about 50% of the cases [25, 26].In our study small necrotic foci were also found in the liver. Fibirnous pericarditis, hepatitis and air sacculitis in experimentally produced *E. coli* infection is also reported [20]. The liver was markedly enlarged and covered by gelatinous material. Various forms of *E. coli* infection cause 8-17% mortality in the affected birds. Fibrinous pericarditis followed by air sacculitis, septicemia, perilobular hepatitis and polyserositis has also been reported [1-5].

In present study, after appearance of clinical signs of colibacillosis birds of all the groups were treated as described above. Mortality in group A was lowest (5.71%), mortality in group B and C was 22.86 and. 17.14%, respectively, while mortality in group D was highest (40%). Least mortality was recorded in group A which was medicated with combination of enrofloxacin plus colistin sulphate. This trail showed that combination of enrofloxacin and colistin is highly effective against avian colibacillosis. FCR of broiler chicks kept in the group A, B, C and D were 2.45, 2.51, 2.65 and 2.89 respectively. FCR increased markedly in group D which were infected with *E. coli* but were not treated. The increased FCR in group D might be due to the anorexia which might have caused low body weight gain.

**Authors' contributions**

Conceived and designed the experiments: T Ahmad & Ghulam Muhammad, Performed the experiments: T Ahmad, Analyzed the data: T Ahmad, G Muhammad, A Sharif & A Shakoor, Contributed materials / analysis / tools: G Muhammad, A Sharif, A Shakoor, M Nadeem & M Rizwan, Wrote the paper: T Ahmad, A Sharif, M Nadeem & Muhammad Rizwan

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