SHORT COMMUNICATION

# Cultural Isolation, Identification, and Antibiogram of *Escherichia coli* from Commercial Layers Affected with Egg Peritonitis

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## Abstract

The present study was conducted on eight commercial layer flocks affected with egg peritonitis (swab samples from oviduct and exudate from the peritoneal cavity) for cultural isolation, identification, biochemical characterization, and antibiogram of *Escherichia coli* (*E. coli*). All the twenty-four samples from 8 commercial layer flocks revealed colonies with typical morphology and biochemical characteristics of *E. coli*. Antibiogram study against seven different antibiotics was conducted by disc diffusion method. The results revealed the highest (100%) sensitivity to antibiotic ceftriaxone, levofloxacin, and tetracycline, followed by amikacin (75.00%) and cefotaxime (62.50%), while the organisms were highly resistant to norfloxacin (75.00%) followed by pefloxacin (62.50%).

**Keywords:** Antibiogram, Commercial layer, Cultural characteristics, Egg peritonitis, *Escherichia coli*.

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## Introduction

Maximum egg production in the poultry industry depends upon a healthy and functional reproductive system of the bird (Keymer, 1980). Any disorder affecting the reproductive system will considerably impact production potential and result in a significant loss. The reproductive disorders in poultry, *viz.,* peritonitis, salpingitis, and impaction of oviduct are described as 'egg peritonitis' (Jordan, 1990). Egg peritonitis is the inflammatory reaction of the peritoneum by the presence of yolk material in the coelomic cavity. Yolk material by itself induces a mild inflammatory response and may be reabsorbed by the peritoneum. Since yolk is the excellent growth medium for bacteria, peritonitis may result from secondary bacterial infection leading to secondary ascites and organ inflammation and causes morbidity, mortality, and reduced egg production in the affected flocks (Zanella *et al*., 2000). The most common infectious bacterial disease is colisepticemia caused by *Escherichia coli***.** It is characterized by multiple organ lesions like air vasculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, yolk sac infection and egg peritonitis. Now a day, there is an increase in the incidence and severity of colisepticemia in layers as well as in broilers and thus impose a major threat on the poultry industry. Therefore, this study aimed to isolate and study the antibiogram of *E. coli* in commercial layer flocks affected with egg peritonitis.

## Materials and Methods

The study was carried out on eight commercial layer flocks affected with egg peritonitis received post-mortem examination at the Department of Pathology, Veterinary College, Anand. For isolation, identification and antibiogram profile of *E. coli*, a total of 24 swab samples (three from each flock) from oviduct and exudate from the peritoneal cavity were collected during autopsy under aseptic precautions with sterile swabs for microbiological analysis.

### Cultural Isolation and Identification of *E. coli*

The samples were streaked on MacConkey agar plates. After 24 hours of incubation the pink coloured colonies were identified and inoculated on Eosin Methylene Blue (EMB) agar. After incubation, bacterial colonies were investigated on the basis of staining, colony morphology, and cultural and biochemical characters of pure isolates.

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### Antibiogram of *E. coli* Isolates

All the twenty four isolates of *E. coli* obtained were subjected to *in vitro* antimicrobial sensitivity test against 7 antibiotics, *viz*., amikacin, ceftriaxone, levofloxacin, cefotaxime, norfloxacin, pefloxacin and tetracycline. The test was carried out by disc diffusion technique of Bauer *et al.* (1966). The code and concentrations of antimicrobial discs used were as mentioned in Table 1.

## Results and Discussion

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

Cultural isolation and identification of *E. coli* was performed on MacConkey agar (MCA) plate. Lactose fermentation by bacteria on MCA plates resulted in appearance of pink coloured colonies (Fig. 1). These colonies were inoculated further on Eosin Methylene Blue (EMB) agar plate. Colonies with greenish metallic sheen on EMB agar plates were tentatively considered to be positive for *E. coli* (Fig. 2). For confirmation, a loopful colony was selected for Gram staining which revealed pink coloured Gram negative bacilli (Fig. 3). Based on cultural characteristics and Gram's staining all the twenty four samples were found positive for *E. coli*.

### Biochemical Characterization of *E. coli* Isolates

All the twenty four *E. coli* isolates obtained were characterized by biochemical tests, *i.e.,* Indol production, Methyl red (MR), Voges-Proskaur (VP) and Citrate utilization test and the results were interpreted according to the Bacteriological Analytical Manual (US FDA: BAM, 2002). All the isolates revealed similar IMViC pattern of + + - - (Fig. 4).

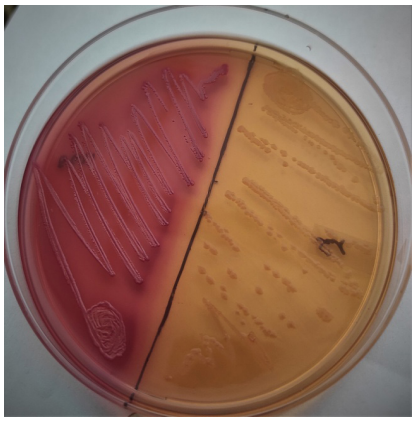

**Fig. 1:** Lactose fermenting pink colour colonies of *E. coli* on MCA

### Antibiogram of *E. coli* Isolates

The *in vitro* antimicrobial drug sensitivity pattern of all the twenty four *E. coli* isolates tested against seven commonly used antibiotics (Fig. 5) revealed that the *E. coli* isolates were highly sensitive to antibiotic ceftriaxone, levofloxacin and tetracycline (100.00%) followed by amikacin (75.00%) and cefotaxime (62.50%), whereas they were highly resistant to norfloxacin (75.00%) followed by pefloxacin (62.50%).

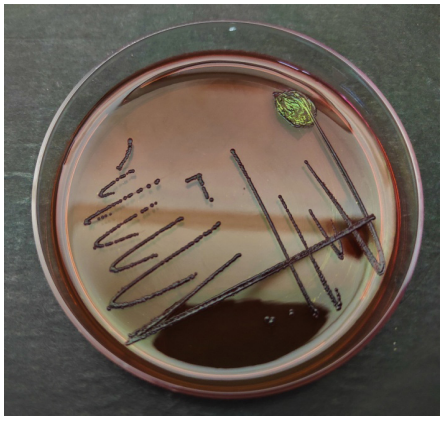

**Fig. 2:** Greenish metallic sheen produced by *E. coli* on EMB agar

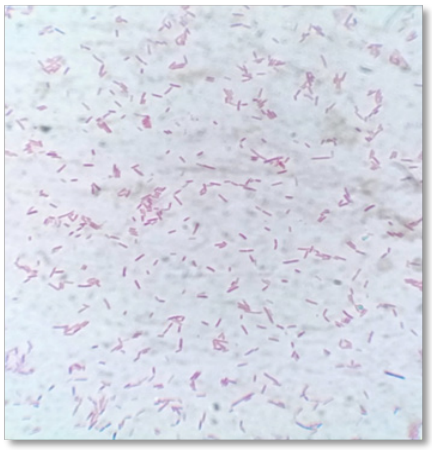

**Fig. 3:** Micrograph revealed pink coloured gram negative bacilli (*E. coli*) (1000x).

**Table 1:** Details of antimicrobial agents and concentration of discs used for antimicrobial susceptibility of *E. coli*

| | | | | *Diameter of zone of inhibition (mm)* | | |
|---|---|---|---|---|---|---|
| *Sr. No.* | *Antibiotics* | *Code* | *Conc. (in µg)* | *Resistant* | *Intermediate* | *Sensitive* |
| 1 | Amikacin | AK | 30 | 10 | 11-15 | 16 |
| 2 | Ceftriaxone | CTR | 30 | 19 | 20-22 | 23 |
| 3 | Levofloxacin | LE | 5 | 16 | 17-20 | 21 |
| 4 | Cefotaxime | CTX | 30 | 22 | 23-25 | 26 |
| 5 | Norfloxacin | NX | 10 | 12 | 13-16 | 17 |
| 6 | Pefloxacin | PF | 5 | 23 | - | 24 |
| 7 | Tetracycline | TE | 30 | 11 | 12-14 | 15 |

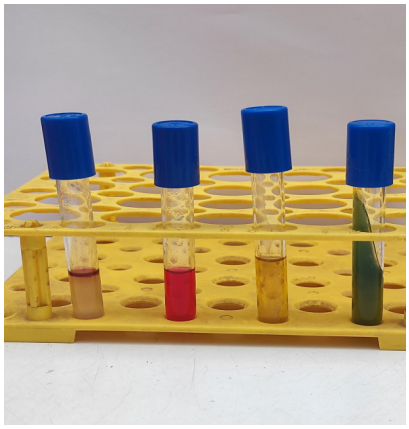

**Fig. 4:** Photograph showing results of IMViC Test for *E. coli* (++--)

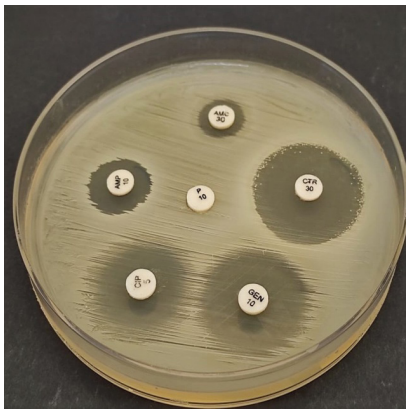

**Fig. 5:** Image showing *in vitro* antibiotic susceptibility pattern of *E.coli* by disc diffusion method

In the present study, all twenty four isolates of *E. coli* from eight flocks were 100% sentitive to ceftriaxone, levofloxacin and tetracycline. Sahoo *et al.* (2012), Abbasi *et al.* (2017) and Amer *et al.* (2018) reported more or less similar sensitivity pattern of ceftriaxone during their study. In contrast to the sesitivity results of present study, Mohamed *et al.* (2014) and Benameur *et al.* (2019) observed 90 to 100 % resistance to levofloxacin, whereas Younis *et al.* (2017) and Thapa and Chapagain (2020) observed resistance of levofloxacin around 50.00% in their study.

In the present study, sensitivity of *E. coli* isolates to cefotaxime was observed to be 62.50%. However, Younis *et al.* (2017) reported only 23.30% sensitivity in their study.

Further, the highest resistance (75.00%) of *E. coli* isolates was observed against norfloxacin in the present study. Mohamed *et al.* (2014) and Jahantigh and Dizaji (2015) also showed comparable 96.00 and 88.00 % resistance to norfloxacin.

The pattern of antibiotic sensitivity using different antibiotics from egg peritonitis affected commercial layer birds has been reported time to time by previous workers. The sensitivity/ resistance pattern of a specific antibiotic was found to be variable by different workers. The antibiotic sensitivity pattern of present study showed ceftriaxone, levofloxacin and tetracycline to be most sensitive antibiotics followed by amikacin, cefotaxime, while norfloxacin and pefloxacin were resistant. It should be noted that antibiotic resistance in poultry is becoming a major issue and unnecessary use of antibiotics should be avoided.

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