

PREVALENCE, BACTERIOLOGY, PATHOGENESIS AND ISOLATION OF *E. COLI* IN BROILERS

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ABSTRACT

This study was conducted to determine the distribution of E. coli in visceral organs from apparently healthy broiler chickens, diseased broiler chickens and freshly dead ones were collected from Kafr-Elsheik Governorate. A total number of 320 chicken organs in winter season and 300 chicken organs in summer season consisting of (80 and 75) from liver, (80 and 75) from heart blood, (80 and 75) from spleen and (80 and 75) from kidney in winter and summer seasons, respectively. Samples were aseptically collected from suspected colibacillosis and subjected to bacteriological and biochemical examination. Processed tissue from each sample was inoculated on nutrient broth and incubated aerobically at 37°C for 12 hours. A loopful from incubated nutrient broth was cultured primarily on MacConkey's agar plates. Positive colonies (pink colonies) were picked up and recultivated on EMB at 37°C for 24 hrs. Presumptive colonies of bacterial agents on media plates were subjected to conventional biochemical characterization. The result of biochemical test identified the E. coli was recovered from 172 chicken organs with an incidence rate (27.74%), the high incidence of E.coli was recovered from liver (50% and 34.6%), followed by fresh heart blood (38.7% and 32%), spleen (22.5% and 12%) and kidneys (18.7% and 12%) in winter and summer seasons, respectively.

INTRODUCTION

Escherichia coli is a rod-shaped, Gram-negative, nonacid fast, uniform staining bacterium that belongs to the *Enterobacteriaceae* family. It is one of the most prevalent pathogenic agents in avians (**Barnes et al., 2008**). *E. coli* is known as one of the most important pathogenic agents causing disease in fowls and mammalians in its both primary and secondary presence are termed Avian Pathogenic *E. coli* (APEC) (**Ewers et al., 2004**).

The natural route of infection for APEC is not clearly defined, although the oral and respiratory routes seem to be major modes of entry (**Harry & Hemsley, 1965**).

A major predisposing factor for systemic APEC infections is stress, which can be induced by a variety of agents or inappropriate husbandry practices (**Barnes & Gross, 1997**). There are numerous other predisposing factors associated with APEC infections such as opportunistic infection, thus underestimating the virulence of APEC. The infection with APEC exacerbates following infection with primary factors such as viral infections like Newcastle disease and Mycoplasmosis, being exposed to the cold weather during breeding and lack of an efficient air conditioning system during the second half of breeding (**Biswaset al., 2006**). Depending on the virulence status of the strain, host status and presence and type of predisposing factors, the infection manifests as an initial septicaemia that is followed by either sudden death or localized inflammation in multiple organs.

Avian Colibacillosis, caused by *Escherichia coli*, is a major infectious disease in birds of all ages and is characterized by a diverse array of lesions such as perihepatitis, airsacculitis and pericarditis, egg

peritonitis, salpingitis, coligranuloma, omphlitis, cellulitis, osteomyelitis /arthritis, septicemia, and death of the birds (*Barnes &Gross, 1997*). This disease has important economic losses in poultry production due to mortality and decrease in productivity of the affected birds (*Otaki,1995*). Recent reports in Western Europe implicate a resurgence of this disease in the poultry industry (*Zanella et al., 2000; Vandekerchove et al., 2004; Jordan et al., 2005*).

MATERIAL AND METHODS

2.1. collection of samples:

A total of 620 tissue samples from poultry chickens organs were collected from different poultry farms in Kafr –Elsheik Governorate, using sterile containers filled with normal saline solution for aerobic culture. The samples were preserved in ice-packs and immediately transferred to the laboratory for microbiological analysis. Samples like liver , heart ,kidney and spleen were collected aseptically from apparently healthy broiler, diseased broiler and freshly dead ones chickens and processed for bacteriological isolation and identification.

2.2. Isolation and identification of *E. coli*

2.2.1 Culture of the samples

Visceral organs were inoculated on nutrient broth and incubated aerobically at 37°C for 12 hours. Suspected *Escherichia coli* colonies were subsequently cultured on MacConkey Agar and incubated at 37°C for 24 hours. After incubation, the MacConkey agar plates were examined for bacterial growth. The isolates of *E. coli* were identified by observing gross colony morphology using MacConkey agar. Discrete colonies of lactose fermenting (pink) and non-lactose fermenting (cream)

bacteria were identified and selected. Lactose positive colonies (pink colonies) were picked up and recultivated on EMB at 37°C for 24 hrs by streak plate method (*Cheesbrough, 1985*) to observe the colony morphology (shape, size, surface texture, edge and elevation, colour, opacity etc). The organisms showing characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies were obtained, metallic green colonies were considered as *E. coli*.

2.2.2. Microscopic identification by Gram's staining method

Gram's staining was performed as per procedures described by *Merchant and Packer (1969)* to determine the size, shape and arrangement of bacteria. The organisms revealed Gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli*.

2.3. Biochemical Characterization of e.coli

2.3.1. Carbohydrate fermentation test

The test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol and incubated for 24 h at 37°C. Acid production was indicated by the color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (*Cheesbrough, 1985*).

2.3.2. Catalase test

A volume of 3 ml of catalase reagent (3% H₂O₂) was taken in a test tube. Single colony from the pure culture of *E. coli* was taken with a

glass rod and merged in the reagent and observed for bubble formation which indicated positive test. Absence of bubble formation indicated negative result (*Cheesbrough, 1985*).

2.3.3. Methyl Red test

Single colony from the pure culture of the test organism was inoculated in 5 ml of sterile MR-VP broth. After 5 days incubation at 37°C, 5 drops of methyl red solution was added and observed for color formation. Development of red or yellow color indicated positive or negative result, respectively (*Cheesbrough, 1985*).

2.3.4. Voges -Proskauer (V-P) test

The test organisms were grown in 3 ml of sterile MR-VP broth at 37°C for 48 h and then 0.6 ml of 5% alphanaphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture. Following well shaking, the broth was allowed to stand for 5-10 minutes to observe the color formation. Development of pink-red color indicated positive result (*Cheesbrough, 1985*).

2.3.5. Indole test

The test organisms were cultured in 3 ml of peptone water containing tryptophan at 37°C for 48 h. One ml of diethyl ether was added, shaken well and allowed to stand until the ether rises to the top. Then 0.5 ml Kovac's reagent was gently run down the side of the test tube to form a ring in between the medium and the ether. Development of brilliant red colored ring indicated positive test (*Cheesbrough, 1985*).

2.3.6. Carbohydrate fermentation test

The test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing five basic

sugars such as dextrose, maltose, lactose, sucrose and mannitol and incubated for 24 h at 37°C. Acid production was indicated by the color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 1985).

2.3.7. Triple sugar iron agar (TSI)

The test organisms were cultured into TSI agar slant by stab or streak method. Yellow slant, yellow butt, presence of gas bubbles and absence of black precipitate in the butt (due to the production of H₂S) indicative of *E. coli* (Cheesbrough, 1985).

2.4. Pathogenicity of *E. coli*:

2.4.1. Motility of *E. coli* isolates:

All isolated *E. coli* showed 100% motilities where they were seen to spread from point of inoculation into the agar as paint brush. The result of motility test picked from the above plates also confirmed the presence of *E. coli*, as the results are positive.

2.4.2. Congo red binding assay:

Strains of *E. coli* were grown in nutrient broth 37°C for 24 hours. A loopful of each culture was grown on Congo red medium and incubated for 24 hours at 37°C. Congo red positive (CR+) *E. coli* was indicated by the development of red colonies. Congo red negative (CR-) *E. coli* did not bind the dye and appeared white colonies. (Berkhoff and Vinal, 1986).

RESULTS AND DISCUSSION

The *E. coli* organisms were identified based on their morphology, cultural, biochemical and sugar fermentation characters as per as shown

in **table (1)**. *Edwards and Ewing (1972) and Cruickshank et al. (1975)* confirmed that. All *E. coli* isolates were able to produce rounded, non-mucoid bright pink colonies (lactose fermenter) on the surface of MacConkey agar medium as in **Figure (1)**. At the same time, the isolates on Eosin Methylene Blue agar (EMB) showed distinctive greenish metallic sheen colonies as in **Figure (2)**. Variation in colony morphology on the EMB agar manifested by the isolates may be due to losing or acquiring some properties by the transfer of host or choice of host tissue such as *E. coli* isolated from cattle on EMB agar showing greenish red colonies with faint metallic sheen (agreed with *Dean (1990) and Dubreuil et al. (1991)*). In Gram's staining, the morphology of the isolated bacteria revealed pink coloured, small rod shaped, non-spore forming Gram negative bacilli (GNB) arranged singly, pairs and groups as shown in **Figure (3)** (*Buxton and Fraser, 1977; Freeman, 1985; Jones, 1987*). Reactions in TSI agar slant revealed yellow slant and butt with gas but no production of hydrogen sulphide gas was observed which agree with *Buxton and Fraser (1977)*. In this study, about 63% of *E. coli* isolates ferment dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. The results of biochemical characters for Catalase, MR and indole test of the *E. coli* isolates were positive but V-P test was negative and that was confirmed by *Buxton and Fraser (1977) and Honda et al. (1982)*. The biochemical test provides confirmation for the presence of *Escherichia coli* in the given tissue samples, as shown in **table (2), figure (4)**. Out of the 620 samples received, 172 isolates were identified as APEC. The prevalence of *E. coli* in the tissue sample was 27.74%. The clinical findings of colibacillosis in broiler birds recorded in this study are in agreement with the reports of *Kaulet al., (1992)* who

reported an outbreak of colibacillosis with clinical signs in broiler. *E. coli* strains that cause infections usually possess one or more virulence properties that may help in establishment of the infection; among these factors is Congo red binding activity. Congo red positive (CR+) *E. coli* colonies are dark red due to binding of the dye as shown in **figure (5)** and colonies that do not bind the dye (CR-) demonstrate a smooth white colonial morphology. Therefore Congo red dye has been associated with pathogenicity of the *E. coli* and that was confirmed by **Vinal (1984), Berkhoff and Vinal (1986) and Gjessing and Berkhoff (1989)**.

Table (1): Identifying Features of *E.Coli*

	Broiler Tissue Samples
1- Colony Characteristics in MacConkey Agar	Large, Circular, Pink, Flat, Moist, Non-mucoid and lactose Lactose-Fermenting
2- Morphology characters	Short rod, single, pair or in short chain
3- Staining Properties	Gram negative

Table (2): Biochemical identification of *E.coli*

Tests	Reaction
Catalase	Bubble formation
Oxidase	-ve reaction
Indole	Red ring formation
Citrate	No color change(no growth)
Methyl red	Bright red (+ve reaction)
Voges-Proskauer	No color change(-ve reaction)
H ₂ S	No H ₂ S production
Urease	Yellow color (-ve reaction)
TSI	Acidic slant/acidic butt
Gelatin hydrolysis	-ve reaction
Nitrate reduction	Red color(+ve reaction)

Fig. (1): Pure Isolates of *Escherichia Coli* on Macconkey Agar Plates

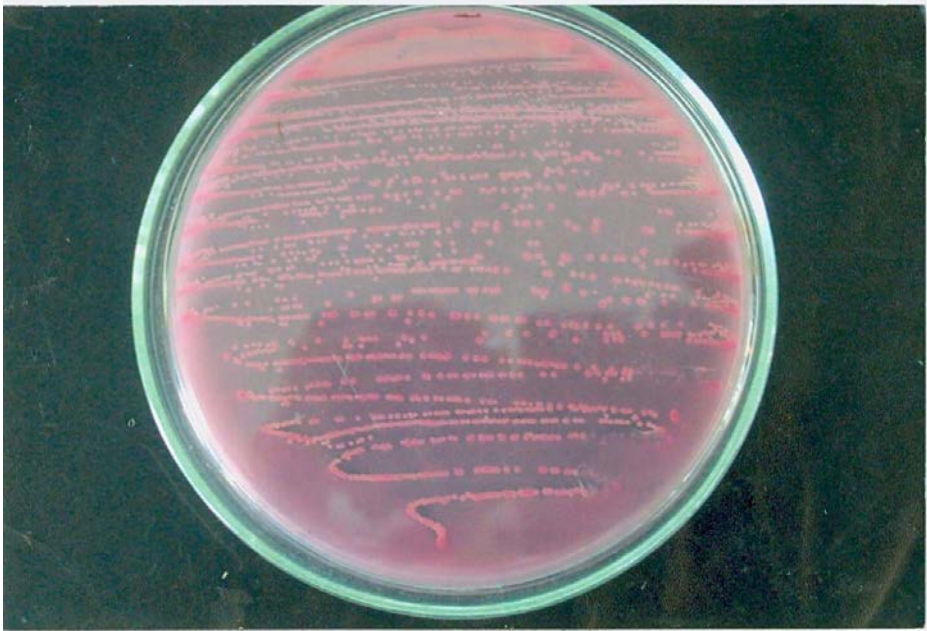


Fig. (2): Pure Isolates of *Escherichia Coli* on EMB Agar

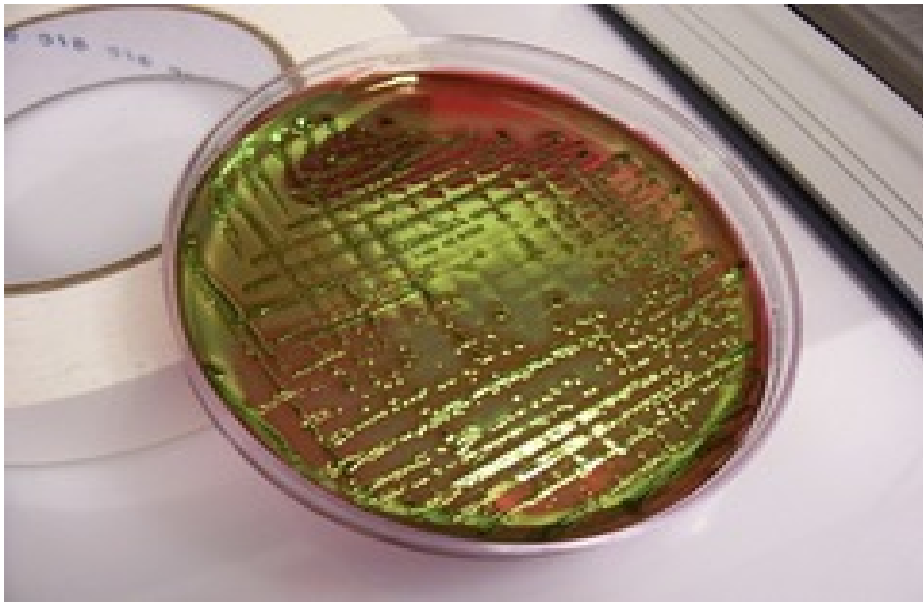


Fig. (3): Photomicrography of Gram Stained Slide

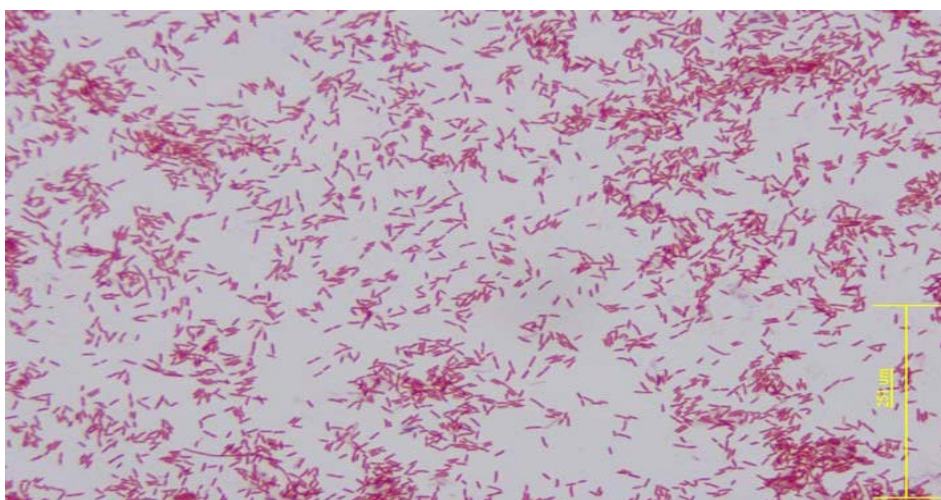


Fig. (4): Biochemical Test (I Mr U C Tsi) Showing Positive I &Mr Test and Negative U & C Test.

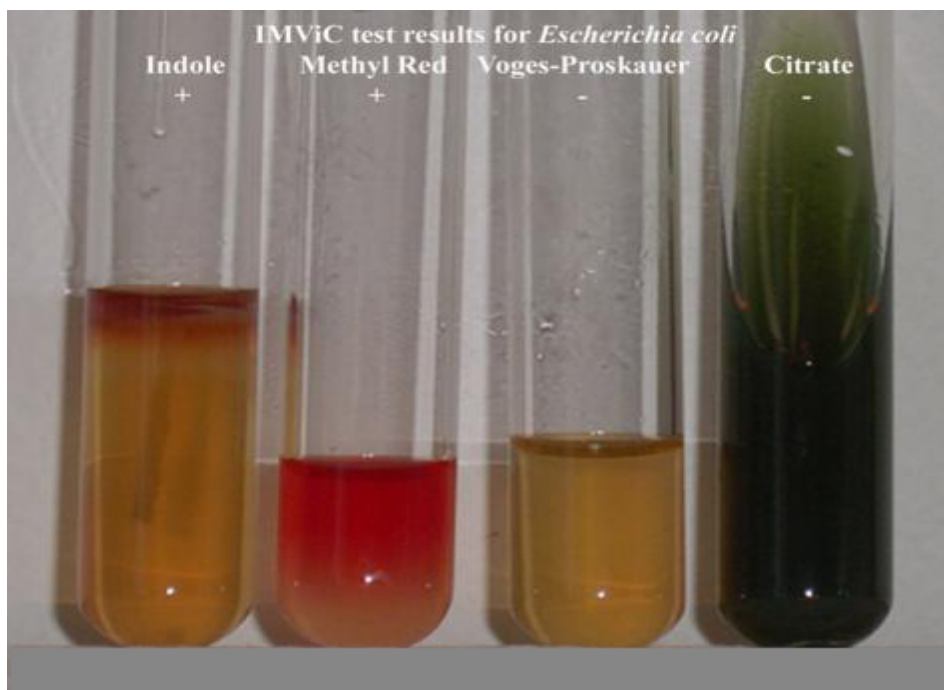
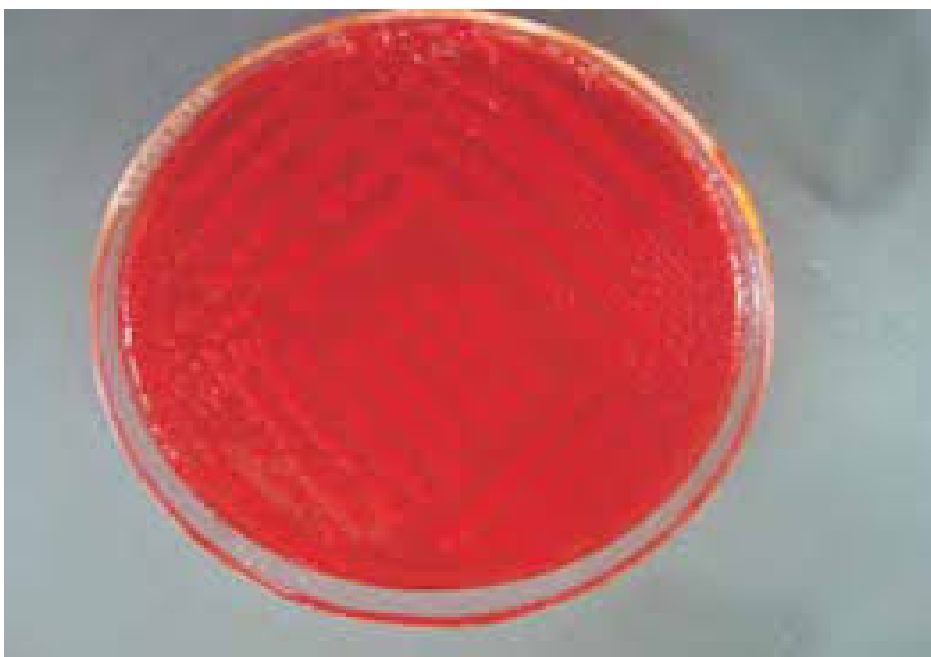


Fig. (5): pathogenic *E. coli* on Congo red media.



In the present study, 113 out of 172 examined *E. coli* strains from chickens(65.6%) were able to bind Congo red dye and appeared as red colonies. In this respect, *EL-ashker (2006)* reported the 70% of *E. coli* isolated from poultry were (CR+), while *Hassan (2009)* reported (CR+) a rate of 100%. *Corbett et al. (1987)* and *Sluggard et al. (1989)* used Congo red dye to differentiate systemically invasive from non-invasive *E. coli*. Also *Harry and Yoder (1989)* used Congo red binding to determine pathogenicity of *E. coli* isolates from broilers poultry. In fact, the nature of Congo red binding phenomenon has not yet been understood but it is suggested by *Berkhoff and vinal (1986)* to be associated with the presence of B-D-glycan in the bacterial cell wall. The pathological lesions of avian colibacillosis are different and wide, and have been reported to be included omphalitis and yolk sac infection, air sacculitis,

pericarditis, salpingitis, peritonitis, enteritis, and colisepticemia. In the present study, omphalitis, pericarditis, fibrinousperihepatitis, dark and congested liver and air sacculitis or were recorded in broiler birds. These lesions in the present study agreed with the earlier reports of **Kaulet al. (1992)**, **Calneket al. (1997)** **Haideret al. (2004)**, **Islam et al. (2004)** and **Khatonet al., (2008)**. Regarding to the higher mortality rates in broilers of selected poultry farms and external traits and symptoms developed in diseased broilers and clinical signs and postmortem lesions in necropsied broilers, caused by *Escherichia coli*. Colibacillosis may be considered as a threat to the broilers industry worldwide. Therefore, these disease problems can be checked by adopting sound management, good sanitation and avoiding overcrowding, using close house system to control humidity, temperature, ventilation and dust for optimal poultry growth and minimal risk of disease outbreak. Attention is to be paid for personal and public hygiene in processing and handling of poultry and poultry products.

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