# *Escherichia coli* as a causative agent in omphalitis in broiler chicks

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Received in 10/7/2013
Accepted in 15/8/2013

**Abstract**

A total of 80 diseased and freshly dead broiler chicks obtained from different farms in Alexandria Governorate were collected. Swabs from unabsorbed yolk sac, enteritis and diarrhea samples were cultured. Bacteriological examination revealed that a total of 46 *E. coli* (57.5%) were recovered from the 80 broiler chicks included, 36 isolates from clinical signs (78.3%) and 10 *E. coli* isolates (21.7%) from freshly dead chicks. The most common serotypes in order of frequency were 15 (32.6%) *E. coli* ($O_{111}$: $K_{58}$) and other 31(67.4%) *E. coli* not include in poly (I, II, III) untypable.

The pathogencity test revealed the virulence of isolated *E. coli* (from clinical sings) on chicks experimentally infected with mortality rate of (80.0%). The postmortem examination of inoculated chicks was unabsorbed yolk sac, dark red distended and/or hyperemic with their content were yellow and yellow brown, putrefactive and offensive odour, septicemia and hemorrhages on serosal surface of visceral organs.

Antibacterial susceptibility testing showed that *E. coli* isolates were sensitive to Ciprofloxacin 5µg (CIP 5)**,** Gentamicin10 µg (GN 10), Doxycycline 30 µg (DO 30) and Colistin 10 µg (CT 10), this may be useful in treatment of Yolk sac infection.

***Key words:*** *Escherichia coli, Omphalitis, broiler chicks.*

## Introduction

Yolk sac infection (YSI) is a major cause of mortality in broilers during their first week of life **(Jordan, 1990)**. The mortality rate is about 5-10%; however the infection may cause a much higher mortality in a batch of chicks in the first week of life **(Wray *et al.,* 1996)**. Yolk sac infection occurs mainly due to bacterial contamination of the eggshell at the broiler breeder farm, shortly after the egg is laid, while the cuticle is still moistened **(Saif *et al.,* 2003)**.

The contamination takes place as a consequence to certain factors including lack of hygiene in the nests, presence of eggs on the floor, incubation of dirty eggs or eggs with eggshell defects, and collection of dirty and clean eggs at the same time **(Mosqueda and Lucio, 1985)**. Poor fertile egg storage conditions, poor egg disinfection, and humidity levels during incubation may also promote egg contamination **(Banis, 1979)**.

*Escherichia coli* O-groups were isolated from diseased and dead chicks suffering from colisepticemia, yolk sac infection or omphalitis **(Dwivedi, 1979; Burkanova, 1970; Azzam, 1983; El Said, 1987; Osman, 1992 and Giovanardi *et al.,* 2005).**

*Escherichia coli* is a normal inhabitant of the intestinal tract of birds, these organisms are capable of producing diseases under the influence of predisposing factors, like inadequate and faulty ventilation, overcrowding, thirst and extremes of temperature. Consequently, losses due to *E. coli* infection occur as a result of high mortality during rearing and reduced weight

gain **(Kaul *et al.,* 1992)**.
Incidence of yolk retention and yolk sac infection was reported as 9% and 8.9% in chicken by **Rathore *et al.,* (1985)** and **Bhattacharjee *et al.,* (1996)**, respectively.
Different types of bacterial agents are attributed for causation of yolk sac infection/ omphalitis. *Escherichia coli* was frequently the main one involved. Its isolation from yolk sac infection was reported by **Anonymous (2000).**
*Escherichia coli* O126 and O111 were the prevalent strains contaminated egg room, setter, hatchery, unhatched egg (dead in shell embryo) and newly hatched chicks **(Al-khalaf *et al.,* 2010).**
*Escherichia coli* were serotyped from moribund birds, 1-7 week of age, with different pathological conditions as perihepatits, enteritis, airsaculitis, yolk sac infection, pnemonitis and pericarditis, predominately O11, O79 and O111 accounting for 26.15% **(Sharada *et al.,* 2010)**.

**Aim of the work.**
This study was conducted to isolate and identify *Escherichia coli* from omphalitis broiler chicks, to determine its pathogenicity by biochemical and serological tests, as well as to choose the drug of choice by making antibiotic sensitivity test.

**Material And Methods**
**Sampling.**
A total number of 80 broiler chicks within the first week of age, including 60 chicks suffering from clinical signs of unabsorbed yolk sac, omphalitis, enteritis and diarrhea clinical signs and 20 freshly dead chicks with the same lesions, were collected from different broiler farms located at Alexandria Governorate for isolation and identification of *Escherichia coli* causing omphalitis in broiler chicks.
**Material**
- Media used for *Escherichia coli* isolation: according to **Oxoid manual (1982).**
- Media used for biochemical reactions: according to **Cruickshank *et al.,* (1975)** and **Oxoid manual (1982).**
- Media used for pathogenicity of *E. coli*: according to **Berkhoff and Vinal (1986).**
- Standard biochemical reagents were used for identification of the bacterial isolates according to **Carter and Cole (1990).**
- Material used for antibiogram determination: according to **Oxoid manual (1982**).

**Methods.**
**1- Bacteriological isolation of *E. coli*.**
Swabs (80) from broiler chicks which suffering from unabsorbed yolk sac, omphalitis, enteritis and diarrhea were collected aseptically in nutrient broth and transported in an ice box to the laboratory as soon as possible, then inoculated into *Escherichia coli* broth tubes and then subcultured onto MacConkey's agar and Eosin Methylene blue agar. The inoculated broth and the streaked agar medium were incubated at 37°C for 24 hours. Pure colonies were picked up and preserved on nutrient agar slope for further morphological, biochemical and serological identification according to **Crucickshank *et al.,* (1982)** and **Speck (1984).**
**2- Bacterial identification.**
The identification of the yielded isolates was performed using standard bacteriological and biochemical procedures as described by **Carter and Cole (1990)** and **Quinn *et al.,* (2002),** those colonies suggestive for *E. coli* were confirmed serologically.
**Biochemical identification by conventional methods.**
The following tests: Oxidase test, Catalase test, IMVC {Indole test, Methyl red test (MR), Voges-Proskauer test (VP) and Citrate utilization test}, TSI (Triple Sugar Iron test), Urea hydrolysis test, Hydrogen sulphide test, as well as fermentation of glucose, lactose and sucrose by change in butt and slants used for identification of isolates **(Gilchrist, 1995; Gray, 1995** and **Farmer, 2003)**.
**Serological identification of *E. coli* isolates.**

Was carried out in the serology unit in Animal Health Research Institute, Dokki, Giza, Egypt.
**Polyspecific products.**
Anti-coli I, Anti-coli II and Anti-coli III***Esche-***

***richia coil* antisera.** It was used for serotyping of *E. coli* according to somatic (O) and capsular (K) antigen. Antisera used for serotyping of *E. coli* isolates: (SIFIN Institute Berlin, Germany).

- **Anti-coli I:** ($O_{26}$:$K_{60}$); ($O_{44}$:$K_{74}$); ($O_{114}$:$K_{90}$); ($O_{125}$:$K_{70}$); ($O_{142}$:$K_{86}$) and ($O_{158}$:K-).
- **Anti-coli II**: ($O_{55}$:$K_{59}$); ($O_{86}$:$K_{61}$); ($O_{91}$:K-); ($O_{111}$:$K_{58}$); ($O_{119}$:$K_{69}$); ($O_{126}$: $K_{71}$); ($O_{127}$: $K_{63}$) and ($O_{128}$: $K_{67}$).
- **Anti-coli III:** ($O_{25}$:$K_{11}$); ($O_{78}$:$K_{80}$); ($O_{103}$:K); ($O_{118}$: K-); ($O_{124}$: $K_{72}$); ($O_{145}$: K-); ($O_{157}$: K-) and ($O_{164}$:K-).

All *E. coli* isolates were serologically typed by slide agglutination test using standard polyvalent and monovalent *E. coli* Antisera. Fresh bacterial cultures of 24 hrs colonies onto TSA medium were used according to **Lee *et al.*, (2009)**.

**Virulence Assay of *E. coli.***

**a) Haemolysis assay.**

*Escherichia coli* isolates were propagated on blood agar base supplemented with 5% sheep blood. Blood agar plates then incubated at 37° C for 24 hrs and colonies producing clear zones of haemolysis were recorded as hemolysin positive **(Vidotto *et al.*, 1990)**.

**b) Congo red Binding Assay.**

The medium used for determination of Congo red binding of the isolates was prepared according to **Berkhoff and Vinal (1986**). Trypticase soya agar was supplemented with 0.003% Congo red dye (Sigma) and 0.15% bile salts. Each isolate was cultured on a separate plate and incubated at 37°C for 24hrs. After 24hrs incubation, the cultures were left at room temperature for 48hrs Invasive *E. coli* was identified by their ability to take up Congo red dye. The positive isolates produced red colonies. The negative isolates appeared as colorless.

**c) Studies on the pathogenicity of the isolated organisms in chicken.**

The experiment was performed to study the pathogenicity of the isolated microorganisms including *E. coli* ($O_{111}$:$K_{58}$).

A total of 25 chicks (1-2 week old) apparently healthy obtained from commercial chicken farms in Alexandria Province were used in the pathogenicity and experimental studies. The chicks were kept in cages and observed for a period a week. A random samples of 5 chicks were slaughtered and exposed to post-mortem, parasitology and bacteriological examination, which proved their healthy status and free from diseases and other chicks were classified into 2 groups Each group contain 10 chicks. Ten isolated cultures of each *E. coli* was collected by centrifugation and resuspended in a saturated solution of $NaHCO_3$ in water. The first group was inoculated by oro-gastric with ($4x10^{10}$/CFU/ml) of *E. coli* while the control group was inoculated with sterile normal saline **(Cantey and Blake, 1977)**. During the observation period (one month), clinical signs, PM lesions were recorded and trials for reisolation of inoculated strains were conducted.

**Antimicrobial sensitivity for the *E. coli* isolates.**

The susceptibility of the bacterial isolates that obtained to the systemic antimicrobial agents most commonly used in poultry industry in Alexandria district was determined, according to the Bauer Kirby procedure **(Bauer *et al.*, 1966)** by the agar - disc diffusion test by using discs of Colistin 10 µg (CT 10), Gentamycin 10 µg (GN 10), Thiamphenicol 30 µg (TP 30), Lincomycin 2 µg (L 2), Doxycycline 30 µg (DO 30), Trimethoprim/Sulphamethoxazole (1.25/23.75) µg (SXT 25), Erythromycin 15 µg (E 15), Ciprofloxacin 5 µg (CIP 5), Amoxicillin 25 µg (AX 25) and Tetracycline 30 µg (TE 30) on Muller-Hinton agar plates. Following 18-24 hours of aerobic incubation, the plates were examined and the diameter of the zone of inhibition was measured by a ruler. The zone diameters were expressed as resistant, intermediate or susceptible according to **Carter and Cole (1990).**

## Results

Results showed that 46 infection with *E. coli* with incidence of (57.5%) was detected, of these 36 (78.3%) isolates from clinical sings and 10 (21.7%) *E. coli* isolates from freshly dead **(Table, 1)**.

**Table (1).** Incidence of bacteria isolated from 80 samples (Clinical signs and freshly dead chicks.

| No. of chick samples (80) | *E. coli* | |
|---|---|---|
| | No. | % |
| Clinical signs (60) | 36 | 78.3 |
| Freshly dead (20) | 10 | 21.7 |
| Total (80) | 46 | 57.5 |

**Results of serological identification of 46 *E. coli* isolates.**

The most common serotype in order of frequency was *15 E. coli* ($O_{111}$: $K_{58}$) with incidence of (32.6%), while 31 other serotypes of *E. coli* were not included in poly (I, II, III) with incidence of (67.4%).

The result of detection of virulence of *E. coli* by using Congo red binding assay and haemolytic activity showed that 11 (73.3%) of *E. coli* ($O_{111}$: $K_{58}$) isolates (pathogenic) out of 15 *E. coli* ($O_{111}$: $K_{58}$) isolates were Congo red positive (+ve CR), and 4 (36.7) *of E. coli* (non pathogenic) were Congo red negative (-ve CR), while the haemolytic activity studies revealed that 10 (66.7%) out of 15 *E. coli* isolates were positive for haemolytic activity and 5 (33.3%) isolates of *E. coli* gave non-haemolytic activity **(Table, 2)**.

**Table (2).** The result of 15 *E. coli* ($O_{111}$:$K_{58}$) isolated from each clinical signs and freshly dead chicks on Congo red Binding Assay and Blood Agar.

| Samples | No. of isolates | Congo Red Binding | | | | Haemolysis assay | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | | Negative | | Positive | | Negative | |
| | | No. | % | No. | % | No. | % | No. | % |
| Clinical signs | 10 | 7 | 70.0 | 3 | 30.0 | 6 | 60.0 | 4 | 40.0 |
| Freshly dead | 5 | 4 | 80.0 | 1 | 20.0 | 4 | 80.0 | 1 | 20.0 |
| Total | 15 | 11 | 73.3 | 4 | 26.7 | 10 | 66.70 | 5 | 33.30 |

**Table (3)** illustrated the results of experimental infection of the susceptible chicks and showed that the isolated strains were pathogenic with mortality rates of 80.0%. The clinical symptoms and post-mortem pictures of inoculated chicks showed signs of putrefactive and offensive odour septicemia including peritonitis, pericarditis, peticheal and ecchymotic hemorrhages on serosal surface of visceral organs associated with infection with *E. coli* similarly with that showed by the examined diseased chicks in the present study.

**Table (3).** The results of pathogenicity of *E. coli* ($O_{111}$: $K_{58}$) isolates from clinical signs chicks.

| Groups | No. of experimentally infected chicks | Type of inoculation | Route of infection | Dose of inoculums | Daily deaths post infection | | | | | | Total No. of deaths | No. of survivors | Mortality rate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Day[d]/Number of deaths | | | | | | | | |
| | | | | | 1-4 | 14 | 15 | 16 | 17 | 18-30 | | | |
| Group ١ | 10 | *E. coli* $O_{111}$:$K_{58}$ | Orally | $4x10^{10}$/ CFU/ml | 3 | 2 | 1 | 1 | 1 | 0-0 | 8 | 2 | 80% |
| Group ٢ (Control) | 10 | Normal saline | | 10ml | 0 | ٠ | ٠ | ١ | ٠ | ٠ | 1 | 9 | 10% |

[d] days after 1st day of infection.

**Table (4)** showed the pattern of antibiotic susceptibility of the most prevalent intestinal pathogens was done in vitro and the obtained data revealed that *E. coli* isolates were sensitive to Ciprofloxacine 5 µg (CIP 5), 5µg, Gentamycin 10 µg (GN 10), Doxycycline 30 µg (DO 30), Colistin 10 µg (CT 10), Tetracycline 30 µg (TE 30) while was resistant to Amoxicillin 25 µg (AX 25), Thiamphenicol 30 µg (TP 30). Lincomycin 2 µg (L 2). Trimethoprim/ Sulphamethoxazole (1.25/23.75) µg (SXT 25). Erythromycin 15 µg (E 15).

**Table (4).** The result of antibiotic susceptibility of *15 E. coli* ($O_{111}$:$K_{58}$) isolated from examined samples.

| Antibacterial agents | Code | Concentration per disc in µg | No of tested *E. coli* ($O_{111}$:$K_{58}$) isolates | susceptibility *E. coli* isolates test | | | |
|---|---|---|---|---|---|---|---|
| | | | | Sensitive | | Resistance | |
| | | | | No. | % | No. | % |
| Ciprofloxacin | CIP | ٥ | ١٥ | 14 | 93.3**** | 1 | 6.7 |
| Gentamycin | GN | ١٠ | | 13 | 86.7**** | 2 | 13.3 |
| Doxycycline | DO | 30 | | 12 | 80**** | 3 | 20 |
| Colistin | CT | 10 | | 10 | 66.7*** | 5 | 33.3 |
| Tetracycline | TE | 30 | | 8 | 53.3** | 7 | 46.7 |
| Lincomycin | L | 2 | | 5 | 33.3** | 10 | 66. |
| Amoxicillin | AX | 25 | | 4 | 26.7** | 11 | 33.3 |
| Thiamphenicol | TP | 30 | | 0 | 0* | 13 | 100* |
| Sulphamethoxazole (1.25/23.75) | SXT | 25 | | 0 | 0* | 15 | 100* |
| Erythromycin | E | 15 | | 0 | 0* | 15 | 100* |

**** = (highly sensitivity) ***= (Intermediate sensitivity)
**= (Low sensitivity) *= (Resistance).

## Discussion

Yolk sac infection is an economically important disease since it increases during the first week of age mortality and causes poor weight gain. In addition, birds that survive to YSI show poor carcass quality **(Cortes *et al.*, 2004).**

The diseased chicks, involved in this study associated with omphalitis, appeared depressed with distended abdomens and suffering from diarrhea and dehydration they were identical with those mentioned by **Saif *et al.*, (2003).**

*Escherichia coli* is considered as a member of the normal microflora of the poultry intestine, but certain strains, such as those designated as Avian Pathogenic *E. coli* (APEC), spread into various internal organs and cause colibacillosis characterized by systemic fatal disease **(La Ragione and Woodward, 2002)**.

*Escherichia coli* is one of the major pathogen responsible for various types of disease conditions in poultry leading to economic loses to poultry industry **(Sharada *et al.*, 2010)**.

In the present study results of bacteriological examination of 80 broiler chick samples (including 60 clinical signs and 20 freshly dead) which suffering from unabsorbed yolk sac, omphalitis, enteritis and diarrhea illustrated in **Table (1)** which revealed that 46 infection with *E. coli* with incidence of (57.5%), of these 36 isolates from clinical sings with incidence of (60.0%), and 10 *E. coli* isolates with incidence of (50.0%) from freshly dead. The high incidence *of E. coli* (57.5%), isolates agreed with **Mahmoud and Moussa (2000)** and **Abeer (2004)** who isolated *E. coli* with incidences of 60% and 55.6%, respectively. While our results were lower than that obtained by **Salman (1999)** and **Farghaly (2000)** who recovered 67.2% and 72.9% isolation *E. coli* rate, respectively from chicken samples.

A large number of different *E. coli* types are present, obtained via horizontal contamination from the environment, more specifically from other birds, faeces, water **(Dho-Moulin and Fairbrother, 1999).**

The serological identification showed that the most common serotypes in order of frequency were *15 E. coli* ($O_{111}$: $K_{58}$) with incidence of (32.6%), while 31 other serotypes of *E. coli* were not included in poly (I, II, III) with incidence of (67.4%). These results agreed with that recorded by **Abd El-Latif (1995)** and **Alkhalaf *et al.*, (2010)** who found that from 88 isolated *E. coli* from contaminated hatcheries, *E. coli* O111 was the most prevalent strain isolated with prevalence rate of 14.8% and also isolated 18 (20.5%) untypable *E. coli*,

While these results disagreed with that reported by **El-Atrebe (1982)** who mentioned that the prevalent strains of *E. coli* were O78 and O125. Also, **Abd El-Galil *et al.*, (1984)** reported that the most prevalent serotypes of *E. coli* were O125, O78, O11 and O55. In addition, **Shalaby and Abd El-Hamid (1987)** who found that the most prevalent serotypes were O78, O128 and O114, **Gross (1994)** and **Chart *et al.*, (2000)** who reported that *E. coli* isolates are pathogenic for poultry commonly belong to certain serogroups, particularly the serogroups O78, O1, and O2, and to some extent O15 and O55. Also **Moawad *et al.*, (2009)** recovered eight *E. coli* isolates from examined diseased broiler chicken and serotyped as the following: 3 isolates (O114: K90), 2 isolates (O26: K60), and one isolate (O91: K-). The prevalence of serotype isolation of *E. coli* varied, this may be attributed to the locality, environmental condition of isolation and personal skill for isolation and identification.

The result of detection of virulence of *E. coli* by using Congo red binding assay and haemolytic activity are shown in **Table (2),** respectively. The result of Congo red assay showed that 11 (73.3%) of *E. coli* ($O_{111}$: $K_{58}$) isolates (pathogenic) out of 15 *E. coli* ($O_{111}$: $K_{58}$) isolates were Congo red positive (+ve CR), and 4 (36.7) *of E. coli* (non pathogenic) were Congo red negative (-ve CR), this result agreed with that recorded by **Berkhoff and Vinal (1986)** who reported a strong correlation between expression of Congo red phenotype and virulence in avian E. coli and suggested that it was associated with the presence of B-D-glucan in bac-

terial cell wall and by **Sharada *et al.*, (2010**) who recovered 95.38% positive Congo red *E. coli* isolates, and disagreed with **Yoder (1989)** who mentioned that Congo red binding did not correlate well with pathogenicity.

While the haemolytic activity studies revealed that 10 (66.7%) out of 15 *E. coli* isolates were positive for haemolytic activity and 5 (33.3%) isolates of *E. coli* gave non-haemolytic activity and these results like that reported by **Vidotto *et al.*, (1990); Style and Flamer (1991)** who suggested that there is a positive association between the (+ve CR) *E. coli* and diarrhea cases. **Okerman (1999)** reported that production of haemolysin by *E. coli* considered as an important virulence factor in *E. coli* infection while disagreed with **Osman *et al.*, (1989)** who attributed heavy mortality in chicks due to non-haemolytic strains of *E. coli*, also **Wooly *et al.*, (1992a)** found that there is no relationship between virulence of *E. coli* and their production of haemolysin and **Sharada *et al.*, (2010**) who said that none of the isolates recovered tested positive for haemolysis production.

Results of experimental infection of the susceptible chicks illustrated in **Table (3)** showed that the isolated strains were pathogenic with mortality rates of 80.0%. The clinical symptoms and post-mortem pictures of inoculated chicks were similarly with that showed by the examined diseased chicks in the present study and this agrees with that reported by **Saif *et al.*, (2003),** who recorded that the yolk sacs were dark red distended and/or hyperemic with their content was yellow, yellow brown and watery. While **Jordan (1990)** reported signs of putrefactive and offensive odour septicemia including peritonitis, pericarditis, peticheal and ecchymotic hemorrhages on serosal surface of visceral organs associated with infection with *E. coli.* A correlation between the chick mortality and the frequency of isolation of *E. coli* was reported by **Cortes *et al.*, (2004**).

**Table (4)** showed that the pattern of antibiotic susceptibility of the most prevalent intestinal pathogens was done in vitro and the obtained data revealed that *E. coli* isolates were sensitive to Ciprofloxacine 5 µg (CIP 5), 5µg, Gentamycin 10 µg (GN 10), Doxycycline 30 µg (DO 30), Colistin 10 µg (CT 10), Tetracycline 30 µg (TE 30) while was resistant to Amoxicillin 25 µg (AX 25), Thiamphenicol 30 µg (TP 30). Lincomycin 2 µg (L 2). Trimethoprim/ Sulphamethoxazole (1.25/23.75) µg (SXT 25). Erythromycin 15 µg (E 15). These results agreed with **Vakani *et al.*, (1997)** and **Sharada *et al.*, (2010**) while differed from those recovered by **Saenz *et al.*, (2001). Ahmed *et al.*, (2000)** reported that *E. coli* showed high rates of resistance to ampicillin, amoxicillin, cotrimoxazole, tetracycline, sulfonamide, trimethoprim, streptomycin, and carbenicillin among commonwhile, **Abu Shaqra (2000)** reported resistant rates of *E. coli* isolates to tobramicin, ceftriaxone and gentamicin as 7%, while the resistance varied from 9-18% for amikacin, ciprofloxacin, norfloxacin, nalidixic acid and cefuroxime.

## References

**Abd El-Galil, Y.; El-Bakry, M.; And Ammar, A. (1984).** Bacterial causes of early chicks mortalities in Sharkia Governorate. Proceeding of 17 World Poultry Congress Helsinki, 557.

**Abd El-Latif, M.M. (1995).** Bacterial causes of lowering fertility, hatchability and early embryonic deaths in balady hatcheries in Dakahlia Governorate. M. V. Sc. Thesis (Microbiology), Fac. Vet. Med. Zagazig University.

**Abeer, E.A. (2004).** Bacterial causes of early mortality in broilers chicken. M. V. Sc. Thesis (Microbiology) Fac. of Vet. Med. Tanta University.

**Abu Shaqra, Q. (2000).** Occurrence and antibiotic sensitivity of *Enterobacteriaceae* isolated from a group of Jordanian patients with community acquired urinary tract infections. Cytobios; 101: 15-21.

**Ahmed, A.A., Osman, H., Mansour, A.M., Musa, H.A., Ahmed, A.B., Karrar, Z., Hassan, H.S. (2000).** Antimicrobial agents' resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. The American Journal of Tropical Medicine and Hygiene; 63: 259-263.

**Al-khalaf, A.N.; Akeila, M.A.; Al-Dubaib, M.A.; Azzam, A.H.; El-Shafey, A.A. and Draz, A.A. (2010).** Bacterial Contamination of Hatcheries. J. Agri. and Vet. Sci., Qassim University, Vol. 2, No. 2, pp. 67-76.

**Anonymous (2000).** Controlling yolk sac infection. Int. Hatchery Pract. 14 (6): 27-28.

**Azzam, A.H. (1983).** Studies on coli septicaemia in poultry in Dakahlia province. M. V. Sc. Thesis, Fac. Vet. Med. Assiut Unversity.

**Bains, B.S. (1979).** A Manual of Poultry Diseases. Roche Publishing, Switzerland.

**Bauer, A.W.; Kirby, W.M.M.; Sherri, J.C. and Turck, M. (1966).** Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

**Berkhoff, H.A. and Vinal, A.C. (1986).** Congo red medium to distinguish between invasive and non-invasive *E. coli* for poultry. Avian Diseases. 30: 117-121.

**Bhattacharjee, P.S., Kundu, R.L.; Biswas, R.K.; Mazumder, J.U.; Hossain, E. and Miah, A.H. (1996).** A retrospective analysis of chicken diseases diagnosed at the Central Disease Investigation Laboratory, Dhaka. Bangladesh. Vet. J., 30 (3-4): 105-113.

**Burkanova, K.K. (1970).** Properties of *E. coli* strains isolated from diseased fowls. Vet. Moscow, USSR 10: 66-68.

**Cantey, J.R. and Blake, R.K. (1977).** Diarrhea due to *E. coli* in the rabbits. A novel mechanism J. Inf. Dis., 135, (3): 454-462.

**Carter, G.R. and Cole, J.R. (1990).** Diagnostic Procedures in Veterinary Bacteriology and Mycology.5th Edition. Academic press, Inc. Diego, California.

**Chart, H.; Smith, H.R.; La Ragione, R.M.; Woodward, M.J. (2000).** An investigation into the pathogenic properties of *Escherichia coli* strains BLR, BL21, DH5α, and EQ1. J. Appl. Microbiol., 89, 1048-1058.

**Cortes, C.R.; Isaies, G.T.; Cuello, C.L.; Floes, J.M.V.; Anderson, R.C. and Campos, C.E. (2004).** Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. Rev. Latinoamericana de MicrobiIologia 46: 12-16.

**Crucickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H. (1982).** Medical Microbiology.12th Ed., Churchill Livingstone Edinburg, London, UK**.**

**Cruichshank, R.; Duguid, J.P.J; Marmion, B.P. and Swain, R.H.A. (1975).** Medical Microbiology 12th ed. Volume II Churchill, Livingstone Edinburgh, London and New York.

**Dho-Moulin, M. and Fairbrother, J.M. (1999).** Avian pathogenic *Escherichia coli* (APEC). Vet. Res., 30, 299-316.

**Dwivedi, P. (1979).** Colibacillosis in poultry. Indian Vet. J. 56 (8): 629.

**El-Atrebe, S.K. (1982).** Studies of the microbial etiology of dead in shell in native hatcheries". Ph. D. Thesis (Microbiology), Fac. Vet. Med., Assiut Univ.

**El-Said, K.M. (1987).** Studies on drug medication in poultry. M. V. Sc. Thesis, Pharmacology, Fac. Vet. Med., Cairo University.

**Farghaly, E.M. (2000).** Some studies on the virulence attributed to *E. coli* isolated from chicken from chicken in Assiut. M. V. Sc thesis (poultry and rabbit diseases), Fac of

Vet. Med., Assuit University.

**Farmer, J.J. (2003).** III *Enterobacteriaceae*: introduction and identification. In: Murray, P. R.; Baron, E. J.; Jorgensen, J. H.; Pfaller, M. A. and Yolken, R. H., editors. Manual of clinical microbiology. 8th ed. Washington: American Society for Microbiology; p. 636–53.

**Gilchrist, M.J.R. (1995).** *Enterobacteriaceae*: opportunistic pathogens and other genera. In Murray, P. R.; Baron, E. J.; Pfaller, M. A.; et al., editors. Manual of clinical microbiology. 6th ed. Washington, D. C. American Society for Microbiology

**Giovanardi, D.; Campagnari, E.; Ruffoni, L.S.; Pesente, P.; Ortali, G.; and Furlattini, V. (2005).** Avian pathogenic *Escherichia coli* transmission from broiler breeders to their progeny in an integrated poultry production chain. Avian Pathol., 34 (4): 313-8.

**Gray, L.D. (1995).** *Escherichia*, *Salmonella*, *Shigella* and *Yersinia*. In Murray, P. R.; Baron, E. J.; Pfaller, M. A.; et al., editors. Manual of clinical microbiology. 6th ed. Washington, D. C. American Society for Microbiology.

**Gross, W.B. (1994).** Diseases due to *Escherichia coli* in poultry. In *Escherichia coli* in Domestic Animals and Humans; Gyles, C. L., Ed.; CAB International Library: Wallingford, UK; pp. 237-260.

**Jordan, F.T.W. (1990).** Poultry Diseases, 3rd Ed., ELBS and Bailliere Tindall, London, U. K., pp: 40.

**Kaul, L.; Kaul, L.P. and Shah, N.M. (1992).** An outbreak of colibacillosis in broiler chicks at an organized poultry farm under semi-arid zone of North Gujarat. Indian Vet. J., 69: 373-374.

**La Ragione, R.M. and Woodward, M.J. (2002).** Virulence factors of *Escherichia coli* serotypes associated with avian colisepticemia. Res. Vet. Sci., 73, 27-35.

**Lee , G.Y.; Janga, H.I.; Hwangb, I.G. and Rheea, M.S. (2009).** Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. Int. J. Food Microbiol., 134 (3): 196-200.

**Mahmoud, A.E.A. and Moussa, H.M. (2000).** Studies on some aerobic bacteria causing of broiler.1st scientific conference for provincial laboratories, Animal Health Research Instit., 15-17 may 2000. Egyptian J. Agri. Res., 78(1): especial Issue, 25-34.

**Moawad; A.A. Mahmoud E.H. and Hammad O.H. (2009).** Bacteria associated with entertitis in broilers in Fayoum Governorate. Kafr-Elsheikh, Vet. Med. J., 3rd Sci. Congress. 10-12 May, pp. (606-635).

**Mosqueda, T.A. and Lucio, M.B. (1985).** Enfermedades communes de la saves domesticas .Universidad Nacional Autonoma de Mexico, pp: 377-381. Mexico, D.F., Mexico.

**Okerman, L. (1999).** Diseases of domestic rabbits. Library of veterinary practice second edition-Blackwell science Ltd. UK.

**Osman, E.; Osman, K.; Mehmut, C. and Ersin, I. (1989).** Haemagglutination, Hydrophobicity, enterotoxigenicity and drug resistance characteristics of avian *E. coli*. Avian Diseases, 33: 631- 635.

**Osman, M.M. (1992).** Studies on bacterial causes of early poultry mortality in Sharkia Governorate. M. V. Sc. Thesis (Microbiology), Fac. Vet. Med., Zagazig University.

**Oxoid manual (1982).** The Oxoid manual of culture media, ingredients and other laboratory services 5th Ed. Oxoid Limit.

**Quinn, P.J.; Carter, M.E.; Markey, B.K.; Donnelly, W.J.C. and Leonard, F.C. (2002).** Veterinary Microbiology and Microbial diseases. MPG, Book. 1st ed., Bodmin, Cornwall, UK.

**Rathore, B.S.; Singh, R. and Khera, S.S. (1985).** Survey on causes of poultry mortality in India, based on postmortem examinations conducted at ten diagnostic centers. Indian J. Poult. Sci., 20 (2): 135-139.

**Saenz, Y.; Zarazaga, M.; Brinas, L.; Lantero, M.; Ruiz-Larrea, F. and Torres. (2001).** Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. Int. J. Antimicrob. Agents, 18 (4): 353-358.

**Saif, Y.M.; Barnes, H.J.; Fadly, A.M.; Glisson, J.R.; Mcdougald, L.R. and Swayne, D.E. (2003).** Diseases of poultry. 11st Edition CD-Rom version. Iowa State press, USA.

**Salman, A.M. (1999).** Serological and bacteriological studies on *E. coli* in chickens. Ph. D., thesis (Microbiology), Fac. of Vet. Med., Cairo University.

**Shalaby, N.A. and Abd El-Hamid, H.S. (1987).** Microbial agents responsible for embryonic mortalities in hatcheries in Gharbia province. Zagazig Vet. J. XV (2): 165.

**Sharada, R.; Ruban, S.W. and Thiyageeswaran, M. (2010).** Isolation, characterization and antibiotic resistance pattern of *Escherichia coli* Isolated from poultry. American –Eurasian J. of Sci. Res., 5 (1): 18-22.

**Speck, M.L. (1984).** Compendium of Methods for the Microbiology examination of foods 2nd Ed American Public Health Association Washington D.C.

**Style, D.K. and Flamer, K. (1991).** Congo red binding of *E. coli* isolated from cloacae of psitacine birds. Avian Disease (35): 46-48.

**Vakani, P.; Murthy, K.K. and Rao, T.V.J. (1997).** In vitro antibiogram studies of *Escherichia coli* in chicken. Indian Vet. J. 74: 616-617.

**Vidotto, M.C.; Muller, E.E.; DeFreitas, J.; Alfieri, A.A.; Guimaraes, L.G. and Stanos, D.S. (1990).** Virulence factors of Avian *E. coli* Dis. 34: 531-538.

**Wooly, E.R.; Spears, R.K.; Brown, J.; Lisa, K.W. and Dekich, A.M. (1992a).** Characteristics of conjugative plasmid from pathogenic *E. coli*. Avian and Dis. 36: 348-352.

**Wray, C.; Davis, R.H. and Corkish, J.D. (1996).** *Enterobacteriaceae*. In: Jordan, F.T.W. & Pattison, M. Eds.) Poultry Diseases. 4th Edition. Saunders Company Ltd. London.

**Yoder, H.W. (1989).** Congo red binding by *Escherichia coli* isolates from chickens. Avian Diseases, 33: 502-505.