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Original Research Article

# Characterization of *E. coli* and *Salmonella* spp. isolates associated with omphalitis in baby chicks

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## ABSTRACT

Omphalitis is a major cause of increased first week-chick mortality. Omphalitis, navel-yolk sac infection, is a hatchery-born disease, and also known as 'mushy chick disease' or 'navel ill'. It is a common disease of chicks and poults, often artificially hatched chicks, causing high losses in the brooding period, as a bacterium penetrates the porous egg shell. As incubation conditions are suitable for bacterial growth and incubating eggs as well, various bacteria, such as *E. coli,* staphylococci*, Proteus, Clostridium fecali and Pseudomonas* may be involved in the yolk sac infection. The present study aimed to determine bacterial causes of omphalitis through isolation and identification of such pathogens. Therefore, samples from 216 yolk sacs were collected from chicks with unabsorbed yolk materials that could even smell putrid. Among those, 196 (90.7%) were positive; 135 (62.5%) harboured single bacterial strains and 61 (28.2%) had mixed infections. The most prevalent single bacterial isolates were *E. coli* (110 isolates) and *P. aeruginosa* (11 isolates). Meanwhile, the most predominant mixed bacterial strains were *E. coli* with *Salmonella* spp. (16 isolates; 7.4%) and *E. coli* with *P. aeruginosa* (13 isolates; 6%). Other mixed infections were found in low percentages. Most *E. coli* strains were Congo red-positive and non-haemolytic. Different *E. coli* serogroups were serologically identified including $O_{27}$ (4 isolates; 20%), $O_{157}$ (3isolates; 15%), $O_{26}$ (3 isolates; 15%) and one isolate of each of the following; $O_{78}$, $O_6$, $O_{125}$, $O_{44}$, $O_{15}$, $O_{115}$, $O_{25}$, $O_{168}$, $O_{112}$ and $O_{63}$ (each of 5%). Different *Salmonella* serogroups were identified including *S. cremieu* (2 isolates) and one isolate of each of the following *S. enteritidis*, *S. blegdam*, *S. senftenberg*, *S. kingston* and *S. emek*. Isolated bacteria differed in

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susceptibility. The adhesion-encoding genes (crl and fimH genes) of *E. coli* were detected by cPCR. It has been concluded that chicks with omphalitis harboured different pathogens and they are considered a source of infection during the successive days of life in broiler chickens.

## 1. Introduction

Omphalitis is becoming a major factor responsible for the early chick mortality during the first few days after hatching (Reece and Beddome, 1983; Ijaz et al., 1994). It is accounting for large economic losses to the poultry industry. It causes a mortality rate of approximately 5-10%. During the incubation, extraembryonic membranes encircle the yolk substance and constitute the yolk sac, which is attached to the gut of the chick by a yolk stalk. Contents of the yolk sac pre- and post-hatch are transported across the epithelial lining into blood capillaries of the yolk sac.

At the beginning of the day 19 of incubation, yolk sac contents are transported directly into the lumen of the small intestine through the yolk stalk (ductus vitellinus) (Esteban et al., 1991a, b; Jeurissen et al., 1991; Noy et al., 1996). Just prior to the hatching, the yolk sac is pulled from the egg cavity to the abdomen of chick (at the site of navel) as an extension of the intestine. Residual yolk comprises 20-25% of the body weight at hatching, but within the first week of life its size becomes negligible (Ramnoff, 1960). The navel should close properly at the point of hatching or within few hours after hatching, so omphalitis can be defined as a bacterial infection of the navel area, resulting from its failure to the properly closure following the withdrawal of the yolk sac into the abdominal cavity, and the infection occurs due to the entrance of bacteria present in the environment. The infection is aggravated by a poor hygiene in breeding farms and faulty management at the hatchery (Gordon and Jordon, 1982). Other factors, such as yolk contents (fat, water, and other favoured nutrients) encourage the bacterial growth.

Moreover, the yolk sac is maintained at the temperature of the hatcher and then at the chick's body temperature; the ideal temperature for the multiplication of certain bacteria (Anonymous, 2000). The most prevalent bacteria causing yolk sac infection is *Escherichia coli* (Deeming, 1995; Rehman et al., 1996; Anjum, 1997; Sharada et al., 1999). Several bacteria such as *E. coli*, *Salmonella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Streptococcus* spp. have been isolated from the yolk sac of infected birds (Cortes et al., 2004; Iqbal et al., 2006).

The current study aimed to determine the main bacterial causes of omphalitis in baby chicks with determination of virulence factors of the most predominant serotype. Serological identification, antimicrobial susceptibility and the detection of virulence genes using PCR were incorporated.

## 2. Material and methods

### 2.1. Samples

A total of 216 yolk sacs were collected from four breeds of baby chicks (Baladi *n*= 111, Saso *n*= 34, Cobb *n*= 33, and Hubbard *n*= 38) suffering from omphalitis at El-Fayoum and Beni-Suef provinces, Egypt during the period from April 2013 to December 2014. Samples were collected, labeled and transported in an ice box to the Reference Laboratory of the Veterinary Quality control on Poultry production.

### 2.2. Bacteriological examination

#### 2.3.1. Isolation of bacterial agents

Different selective and differential media including MacConkey agar, EMB, SS agar, XLD agar, *Pseudomonas* agar base with C-N supplement, mannitol salt agar and blood agar were used.

#### 2.3.2. Morphological examination

It was done according to Cruickshank et al. (1975).

#### 2.3.3. Biochemical identification

Pure colonies of isolates were biochemically identified according to Finegold and Martin (1982), Koneman et al. (1995), and Quinn et al. (2002).

#### 2.3.4. Detection of virulence factors

Congo red-binding test and haemolytic activity were performed for differentiation of pathogenic and non-pathogenic bacterial isolates and for differentiation of haemolytic and non-haemolytic isolates.

**2.3. Serological typing of isolated bacteria**

**2.3.1. Serological identification of *E. coli***

Isolates that were preliminary identified biochemically as *E. coli* were subjected to serological identification according to Ewin (1986).

**2.3.2. Serological identification of *Salmonella***

The organisms were serotyped according to Kauffmann and Das-Kauffmann (2001).

**2.3. Antibiogram test**

Isolated bacteria were subjected to different antimicrobial agents by the disc diffusion method and evaluated according to Clinical and Laboratory Standard Institute (CLSI) (2013). The following antibacterial agents were used: Florfenicol (30 µg), ciprofloxacin (5 µg), amoxycillin (10 µg), amoxicillin plus clavulanic acid (10 µg), doxycycline hydrochloride (30 µg), cefotaxime sodium (30 µg), gentamycin (10 µg), neomycin (30), sulpha plus trimethoprime (25) and clostin (10 µg). All antimicrobial discs were purchased from Oxoid, UK.

**2.4. Detection of the adhesin-encoding genes of *E. coli* isolates by cPCR:**

Bacterial DNA was extracted from selected colonies using QIAamp DNA mini kit (Qiagen) according to the manufacturer instructions. Two PCR runs were performed according to Ghanbarpour and Salehi (2010) using one of the following primers; *Crl* (For 5'-TTTCGATTGTCTGGCTGTATG-3' Rev 5'-CTTCAGATTCAGCGTCGTC-3'), *fimH* (For 5'-TGCAGAACGGATAAGCCGTGG-3' Rev 5'-GCAGTCACCTGCCCTCCGGTA-3'). The expected product size for Crl and fimH primers are 250 bp and 508 bp, respectively (Table 1).

**Table 1. Oligonucleotide primers sequences (Source: Biobasic, Canada)**

| Target Gene | Primer sequences | Amplified segment (bp) | Reference |
|---|---|---|---|
| *Crl* | TTTCGATTGTCTGGCTGTATG | 250 | Ghanbarpour and Salehi, (2010) |
| | CTTCAGATTCAGCGTCGTC | | |
| *fimH* | TGCAGAACGGATAAGCCGTGG | 508 | |
| | GCAGTCACCTGCCCTCCGGTA | | |

## 3. Results

**3.1. Bacteriological examination**

Out of 216 cases, 196 (90.7%) were positive with bacterial isolates. Bacteriological examination of diseased chicks revealed that 135(62.5%) harboured single bacterial strains and 61(28.2%) had mixed infections (Table 2).

**Table 2. The overall bacterial isolation from chicks with omphalitis**

| Bacterial isolation from chicks | Number | Percentage |
|---|---|---|
| Single isolates | 135 | 62.5 |
| Mixed isolates | 61 | 28.2 |
| Negative isolation | 20 | 9.3 |
| Total | 216 | 100 |

**3.1.1. Isolation frequency of the bacterial strains**

Bacteriological examination of 216 yolk sacs revealed a total of 257 bacterial isolates. The identification of such isolates detected *E. coli* (152; 70.3%), *Pseudomonas aeruginosa* (41; 18.9%), *Salmonella* spp. (25; 11.5%), Coagulase Negative staphylococci *(CNS)* (13; 6%), *Staphylococcus aureus* (10; 4.6%), *Proteus mirabilis* (10; 4.6%), *Klebsiella pneumoniae* (3; 1.3%), *Streptococcus* spp. (2; 0.9%), and *Enterobacter aerogenes* (one isolate; 0.4%) (Table 3).

**3.1.2. Bacterial isolation from different breeds**

The bacterial isolation from different breeds was shown in Table (3).

**3.1.2.1. Single bacterial isolates**

It has been found that out of 216 of examined chicks, 135 (62.5%) birds harbored single isolates. The prevalence of isolated species were 50.92%, 5.10, 2.31, 1.85, 0.93, 0.93, and 0.46% for *E. coli* (110 isolates), *P. aeruginosa* (11 isolates), *Salmonella* spp. (5 isolates), *P. mirabilis* (4 isolates), *S. aureus* (2 isolates), *Streptococcus* spp. (2 isolates), and *K. pneumoniae* (one isolate), respectively (Table 4).

**Table 3. Bacterial species recovered from yolk sacs of different breeds of chicks with omphalitis.**

| Breed / Bacteria | Baladi | | Saso | | Cobb | | Hubbard | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| *E. coli* | 73 | 65.7 | 24 | 70.5 | 19 | 57.5 | 36 | 94.7 | 152 | 70.3 |
| *P. aeruginosa* | 36 | 32.4 | 4 | 11.7 | 1 | 3 | - | - | 41 | 18.9 |
| *Salmonella* | 7 | 6.3 | 2 | 5.8 | 14 | 42.4 | 2 | 5.2 | 25 | 11.5 |
| *CNS* | 10 | 9 | 2 | 5.8 | 1 | 3 | - | - | 13 | 6 |
| *S. aureus* | 8 | 7.2 | 2 | 5.8 | - | - | - | - | 10 | 4.6 |
| *P. mirabilis* | 6 | 5.4 | - | - | 3 | 9 | 1 | 2.6 | 10 | 4.6 |
| *K. pneumonia* | 2 | 1.8 | 1 | 2.9 | - | - | - | - | 3 | 1.3 |
| *Streptococcus* | - | - | 2 | 5.8 | - | - | - | - | 2 | 0.9 |
| *E. aerogenes* | - | - | - | - | 1 | 3 | - | - | 1 | 0.4 |

No. referred to number of infected birds % referred to percentage of infection

**Table 4. The prevalence of single bacterial isolates from chicks with omphalitis.**

| Bacterial isolates | Number | Percentage |
|---|---|---|
| *E. coli* | 110 | 50.93 |
| *P. aeruginosa* | 11 | 5.1 |
| *Salmonella* | 5 | 2.31 |
| *P. mirabilis* | 4 | 1.85 |
| *S. aureus* | 2 | 0.93 |
| *Streptococcus* spp. | 2 | 0.93 |
| *K. pneumonia* | 1 | 0.46 |
| Total | 135 | 62.5 |

**3.1.2.2. Mixed bacterial isolates**

A total of 61 examined chicks contained more than one species of bacteria with an incidence of 28.2%. They identified as *E. coli* with *Salmonella* spp. (16 isolates; 7.4%) and *E. coli* with *P. aeruginosa* (13 isolates; 6%), *E. coli* with *S. aureus* (7 isolates; 3.24%), *E. coli* with *C. N.* staphylococci (4 isolates; 1.9%), 3 *E. coli* ,*C.N.* staphylococci with *P. aeruginosa* (1.38%), *E. coli* with *P. mirabilis* (one isolate; 0.46%), *E. coli, Salmonella* spp. with *P. aeruginosa* (one isolate; 0.46%), *E. coli, P. mirabilis* with *P. aeruginosa* (one ispolate; 0.46%), *P. aeruginosa* with *S. aureus* (one isolate; 0.46%), *P. aeruginosa* with *C.N.* staphylococci (5 isolates; 2.30%), *P. aeruginosa* with *P. mirabilis* (3 isolates; 1.38%), *P. aeruginosa* with *Salmonella* spp. (3 isolates; 1.38%), *P. aeruginosa* with *K. pneumoniae* (one isolate; 0.46%), *P. aeruginosa, K. pneumoniae* with *C.N.* staphylococci (one isolate; 0.46%), and *Salmonella* spp. with *E. aerogenes* (one isolate; 0.46%) (Table 5).

**3.2. Virulence factors in *E. coli***

**3.2.1. Congo red binding assay**

Out of 152 *E. coli* isolates, 151 (99.3%) showed Congo red binding activity and only one (0.7%) isolate was negative (Table 6).

**3.2.2. Haemolytic activity**

Out of 152 *E. coli* isolates, only one (0.7%) isolate revealed beta hemolysis on blood agar and 151 (99.3%) were non-hemolytic (Table 6).

**3.3. Serological typing of isolated bacteria**

Serological typing of *E. coli* and *Salmonella* isolates was indicated in Tables 7 and 8.

**3.4. Antibiogram test**

*In vitro* sensitivity test revealed that 20 isolates of *E. coli*, 7 isolates of *Salmonella* spp., 7 isolates of *P. aeruginosa*, 5 isolates of *P. mirabilis*, 5 isolates *S. aureus*, 3 isolates of *K. pneumoniae*, 2 isolates of *Streptococcus* spp. and one isolate of *E. aerogenes* were tested against different antimicrobial agents, resistant, intermediate or sensitive.

**Table 5. The prevalence of mixed bacterial isolates from chicks with omphalitis.**

| Mixed isolates | Number | Percentage |
|---|---|---|
| *E. coli* + *Salmonella* spp. | 16 | 7.4 |
| *E. coli* + *P. aeruginosa* | 13 | 6 |
| *E. coli* + *S. aureus* | 7 | 3.24 |
| *E. coli* + *C.N.* staphylococci | 4 | 1.9 |
| *E. coli* + *C.N.* staphylococci + *P. aeruginosa* | 3 | 1.38 |
| *E. coli* +*P. mirabilis* | one | 0.46 |
| *E. coli* + *Salmonella* spp.+ *P. aeruginosa* | one | 0.46 |
| *E. coli* + *P. mirabilis* + *P. aeruginosa* | one | 0.46 |
| *P. aeruginosa* + *S. aureus* | one | 0.46 |
| *P. aeruginosa* + *C.N.* staphylococci | 5 | 2.3 |
| *P. aeruginosa* + *P. mirabilis* | 3 | 1.38 |
| *P. aeruginosa* + *Salmonella* spp. | 3 | 1.38 |
| *P. aeruginosa* + *K. pneumoniae* | one | 0.46 |
| *P. aeruginosa* + *K. pneumoniae* + *C.N.* staphylococci | one | 0.46 |
| *Salmonella* spp.+ *E. aerogenes* | one | 0.46 |

**Table 6. Virulence factors of *E. coli* isolated from chicks with omphalitis.**

| Virulence Factors | Positive | | Negative | |
|---|---|---|---|---|
| | Number | Percentage | Number | Percentage |
| Congo red test | 151 | 99.3 | one | 0.7 |
| Hemolytic activity | one | 0.7 | 151 | 99.3 |
| Total | 152 | 100 | 152 | 100 |

### 3.5. Detection of the adhesin-encoding genes of *E. coli* isolates by PCR

Isolates of *E. coli* (20 serotypes) were positive using the specific primers for Curli (Crl) gene and Fim H genes giving specific bands of 250 bp and 508 bp (Figs. 1, 2).

## 5. Discussion

Omphalitis is becoming a major factor responsible for the early chick mortality during the first few days after hatching (Reece and Beddome, 1983; Ijaz et al., 1994). Bacteriological examination revealed that out of 216 cases, 196 (90.7%) was positive. Among the diseased chicks, 135 and 61 harbored bacterial strains as single and mixed infections with an incidence of 62.5% and 28.2%, respectively. The presence of single or multiple bacterial infection inducing omphalitis matched with those obtained by Hussein et al. (2008) who stated that of 140 examined yolk samples, a positive bacterial isolation was obtained from 107 (76.4%), of which 42 (30%) revealed a single bacterial isolate and 65(46%) had mixed bacterial isolates.

Isolation of bacterial strains from 216 yolk sacs revealed that a total of 257 bacterial isolates were recovered. The identification of such isolates revealed were *E. coli* (152; 70.3%), *Pseudomonas aeruginosa* (41; 18.9%), *Salmonella* spp. (25; 11.5%), *Coagulase Negative* staphylococci *(CNS)* (13; 6%), *Staphylococcus aureus* (10; 4.6%), *Proteus mirabilis* (10; 4.6%), *Klebsiella pneumoniae* (3; 1.3%), *Streptococcus* spp. (2; 0.9%), and *Enterobacter aerogenes* (one; 0.4%). Such results agreed with those of Saif et al. (2008) who reported that *E. coli* is the most common contaminant of yolk sacs in chickens and approximately 70% of chicks with omphalitis had such species in their yolk sacs. On the other hand, Iqbal et al. (2006) isolated various species of bacteria including *E. coli* (83.9%), *Proteus* (5.87%), mixed infection (3.59%), *Streptococci* (2.89%), *Klebsiella* (1.79%), *Salmonella* (0.5%), staphylococci (0.5%), and *Pseudomonas* (0.5%) from chicks with omphalitis. Hussein et al. (2008) stated that a total of 191 bacterial strains belonged to different genera were isolated; *E. coli* 83(43.5%), *Enterobacter aerogenes* 31(16.2%), *Staphylococcus aureus* 24 (12.6%),

*Klebsiella pneumoniae* 23 (12%) and 4 species of lower proportions; *Streptococcus* spp., *Proteus mirabilis*, *Bacillus cerus* and *Pseudomonas aeruginosa.*

**Table 7. Serotyping of *E. coli* isolates.**

| Serotype | Number of isolates | Percentage |
|---|---|---|
| *E. coli* $O_{27}$ | 4 | 20 |
| *E. coli* $O_{157}$ | 3 | 15 |
| *E. coli* $O_{26}$ | 3 | 15 |
| *E. coli* $O_{78}$ | 1 | 5 |
| *E. coli* $O_{6}$ | 1 | 5 |
| *E. coli* $O_{125}$ | 1 | 5 |
| *E. coli* $O_{44}$ | 1 | 5 |
| *E. coli* $O_{15}$ | 1 | 5 |
| *E. coli* $O_{115}$ | 1 | 5 |
| *E. coli* $O_{25}$ | 1 | 5 |
| *E. coli* $O_{168}$ | 1 | 5 |
| *E. coli* $O_{112a}$ | 1 | 5 |
| *E. coli* $O_{63}$ | 1 | 5 |
| Total | 130 | 100 |

**Table 8. Serotyping of *Salmonella* spp. isolates.**

| Serotypes of *Salmonella* | Number of isolates |
|---|---|
| *S. cremieu* | 2 |
| *S. enteritidis* | one |
| *S. blegdam* | one |
| *S. senftenberg* | one |
| *S. kingston* | one |
| *S. emek* | one |
| Total | **7** |

In the current study, the most prevalent single bacterial isolates were *E. coli* (110 isolates), *P. aeruginosa* (11 isolates), *Salmonella* spp. (5 isolates), *P. mirabilis* (4 isolates), *S. aureus* (2 isolates), *Streptococcus* spp. (2 isolates) and *K. pneumoniae* (one isolate) with prevalence rates of 50.92, 5.1, 2.31, 1.85, 0.93, 0.93, and 0.46%, respectively. These findings are more or less similar to those obtained by Amare et al. (2013) who found that *E. coli* was the most predominant isolate (51.17%), followed by *Staphylococcus aureus* (23.53%), *Proteus mirabilis* (22.94%) and *Streptococcus* spp. (lower proportion). A total of 61 (28.2%) examined chicks contained more than one species of bacteria; *E. coli* with *Salmonella* spp. (*n*= 16; 7.4%), *E. coli* with *P. aeruginosa* (*n*= 13; 6%), *E. coli* with *S. aureus.* (*n*=7; 3.24%), *E. coli* with *C.N.* staphylococci (*n*=4; 1.9%), *E. coli*, *C.N.* staphylococci with *P. aeruginosa* (*n*=3; 1.38%), *E. coli* with *P. mirabilis* (*n*= one; 0.46%), *E. coli*, *Salmonella* spp. with *P. aeruginosa* (*n*= one; 0.46%), *E. coli*, *P. mirabilis* with *P. aeruginosa* (*n*= one; 0.46%), *P. aeruginosa* with *S. aureus* (*n*= one; 0.46%), *P. aeruginosa* with *C.N.* staphylococci (*n*= 5), *P. aeruginosa* with *P. mirabilis* (*n*=3; 1.38%), *P. aeruginosa* with *Salmonella* spp. (*n*=3; 1.38%), *P. aeruginosa* with *K. pneumoniae* (*n*= one; 0.46%), *P. aeruginosa*, *K. pneumoniae.* with *C.N.* staphylococci (*n*= one), and *Salmonella* spp. with *E. aerogenes* (*n*= one). It has been revealed that *E. coli* was the most predominant in mixed infections (Amare et al., 2013). It was noticed that 151 (99.3%) isolates out of 152 *E coli*-positive samples were able to bind to the Congo red dye. These findings went parallel to those reported by Khalil (2012) and Berkhoff and vinal (1986).

Among 152 *E. coli* isolates, only one (0.7%) isolate gave beta hemolysis on blood agar. These findings are more or less similar to those of Emery et al. (1992), Fantinatti et al. (1994) and Parriera et al. (2002). On the other hand, Khalil (2012) stated that most of *E. coli* isolates causing omphalitis are haemolytic causing alpha hemolysis (82%) and beta hemolysis (18%).

The serological typing of 20 *E. coli* isolates recovered from diseased chicks revealed that out of 20 isolates, 4 (20%) isolates were $O_{27}$, 3 (15%) isolates as $O_{157}$, 3 (15%) isolates as $O_{26}$, one (5%) isolate as $O_{78}$, one (5%) isolate as $O_{6}$, one (5%) isolate as $O_{125}$, one (5%) isolate as $O_{44}$, one (5%) isolate as $O_{15}$, one (5%) isolate as $O_{115}$, one (5%) isolate as $O_{25}$, one (5%) isolate as $O_{168}$, one (5%) isolate as $O_{112a}$, and one (5%) isolate as $O_{63}$. Such results run parallel with those obtained by Amira et al. (2010) who isolated serogroups $O_{27}$ from cloacal swabs of chickens. Similarly, Rezk et al. (2010) isolated 275 *Escherichia coli* strains from diseased chickens obtained from different localities in Ismailia province, Egypt. Serotyped strains (*n*=40) belonged to 10 serovars, $O_{78}$ (*n*=13), $O_{153}$ (*n*=6), $O_{168}$ (*n*=5), $O_{26}$ (*n*=4), $O_{157}$ and $O_{146}$ (*n*=3 each), $O_{20}$ and $O_{114}$ (*n*=2 each), $O_{125}$ and $O_{126}$ (*n*=one each). Salehi and Ghanbarpour (2010) isolated *E. coli* serogroups $O_{6}$, $O_{15}$, $O_{25}$, and $O_{78}$ from oviducts of layer hens with salpingitis. Meanwhile, Kika et al. (2013) isolated *E. coli* serogroups $O_{15}$ and $O_{115}$ from visceral organs of birds with colibacillosis. Heba et al. (2012) isolated *E. coli* serogroups $O_{44}$, $O_{125}$, $O_{26}$, $O_{78}$, $O_{157}$ and $O_{6}$ from chicken and ducks. Guastalli

et al. (2013) serogrouped 44 strains of *E. coli* with high and intermediate pathogenicity levels isolated from livers and intestines from 20 examined chicken flocks from which $O_{15}$ (4.54%) and $O_{112}$, (2.27%). The serological typing of 7 *Salmonella* spp. isolates recovered from diseased chicks revealed that 2 isolates were identified as *S. cremieu*, one isolate as *S. enteritidi*, one isolate as *S. blegdam*, one isolate as *S. senftenberg*, one isolate as *S. kingston* and one isolate as *S. emek*. Such findings were in agreement with those obtained by Mona et al. (2014) who isolated *S. cremieu* from drag swabs and *S. enteritidis* from internal organs of layer chickens in different farms in Egypt. Ezzat et al. (2014) isolated *S. blegdam* from broiler farms in Dakahlia province, Egypt. Furthermore, Al-Nakhli et al. (1999) isolated *S. kingston*, *S. emek*, *S. enteritidis* and *S. senfetenberg* from poultry (heart, liver, spleen, intestine, yolk sac and cloacal swabs) and their surrounding environments.

**Table 9. Antibiogram profile of Enterobacteriaceae isolated from chicks with omphalitis**

| Antimicrobial discs | *E. coli* | | | | | | *Salmonella* spp. | | | | | | *P. mirabilis* | | | | | | *K. pneumoniae* | | | | | | *E. aerogenes* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | | I | | R | | S | | I | | R | | S | | I | | R | | S | | I | | R | | S | | I | | R | |
| | No | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Amoxicillin | 0 | 0 | 0 | 0 | 20 | 100 | 0 | 0 | 0 | 0 | 7 | 100 | 0 | 0 | 0 | 0 | 5 | 100 | 0 | 0 | 0 | 0 | 3 | 100 | 0 | 0 | 0 | 0 | 1 | 100 |
| Amoxicillin /clavulanicaacid | 16 | 80 | 4 | 20 | 0 | 0 | 7 | 100 | 0 | 0 | 0 | 0 | 3 | 60 | 0 | 0 | 2 | 40 | 3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 100 |
| Cefotaxime | 14 | 70 | 1 | 5 | 5 | 25 | 5 | 71.4 | 1 | 14.3 | 1 | 14.3 | 1 | 20 | 2 | 40 | 2 | 40 | 2 | 66.7 | 0 | 0 | 1 | 33.3 | 1 | 100 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 18 | 90 | 1 | 5 | 1 | 5 | 3 | 42.9 | 4 | 57.1 | 0 | 0 | 4 | 80 | 1 | 20 | 0 | 0 | 3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 100 | 0 | 0 |
| Colistinsulphate | 16 | 80 | 1 | 5 | 3 | 15 | 6 | 85.7 | 0 | 0 | 1 | 14.3 | 0 | 0 | 0 | 0 | 5 | 100 | 2 | 66.7 | 0 | 0 | 1 | 33.3 | 1 | 100 | 0 | 0 | 0 | 0 |
| Doxycycline | 2 | 10 | 3 | 15 | 15 | 75 | 5 | 71.4 | 1 | 14.3 | 1 | 14.3 | 0 | 0 | 0 | 0 | 5 | 100 | 1 | 33.3 | 0 | 0 | 2 | 66.7 | 0 | 0 | 0 | 0 | 1 | 100 |
| Florfenicol | 14 | 70 | 1 | 5 | 5 | 25 | 7 | 100 | 0 | 0 | 0 | 0 | 4 | 80 | 0 | 0 | 1 | 20 | 1 | 33.3 | 0 | 0 | 2 | 66.7 | 1 | 100 | 0 | 0 | 0 | 0 |
| Gentamycin | 18 | 90 | 0 | 0 | 2 | 0 | 7 | 100 | 0 | 0 | 0 | 0 | 4 | 80 | 1 | 20 | 0 | 0 | 3 | 100 | 0 | 0 | 0 | 0 | 1 | 100 | 0 | 0 | 0 | 0 |
| Neomycin | 7 | 35 | 8 | 40 | 5 | 25 | 5 | 71.4 | 1 | 14.3 | 1 | 14.3 | 1 | 20 | 2 | 40 | 2 | 40 | 2 | 66.7 | 0 | 0 | 1 | 33.3 | 1 | 100 | 0 | 0 | 0 | 0 |
| Sulphamethoxazole /trimethoprime | 10 | 50 | 0 | 0 | 10 | 50 | 6 | 85.7 | 0 | 0 | 1 | 14.3 | 3 | 60 | 0 | 0 | 2 | 40 | 1 | 33.3 | 0 | 0 | 2 | 66.7 | 0 | 0 | 0 | 0 | 1 | 100 |

S = Sensitive I = Intermediate R = Resistant

**Table 10. Antibiogram test of *Pseudomonas aeruginosa* isolated from diseased chicks.**

| Antibacterial agents | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **Cefotaxime** | 1 | 14.3 | 2 | 28.6 | 4 | 57.1 |
| **Ciprofloxacin** | 7 | 100 | 0 | 0 | 0 | 0 |
| **Colistinsulphate** | 6 | 85.7 | 0 | 0 | 1 | 14.3 |
| **Doxycycline** | 0 | 0 | 0 | 0 | 7 | 100 |
| **Florfenicol** | 1 | 14.3 | 0 | 0 | 6 | 85.7 |
| **Gentamicin** | 7 | 100 | 0 | 0 | 0 | 0 |

Findings of antimicrobial susceptibility revealed that most of the Enterobacteriacae group is susceptible to gentamycine, florfenicol, cefotaxime, ciprofloxacin, and amoxicillin mixed with clavulanic acid, while it is resistant to amoxicillin and doxycycline. Such results were partially similar to those reported by Hussein et al. (2008) whose isolates were susceptible to florfenicol, enrofloxacine and co-trimoxasole, and resistant to amoxicillin, flumequine and tetracycline. The present investigation revealed that the majority of *P. aeruginosa* strains were highly sensitive to ciprofloxacin and gentamycin (100% each) followed by colistinsulphate (85.7%). Such findings were more or less similar to those obtained by Abd el-Gawad et al. (1998) who recorded that *P. aeruginosa* strains isolated from chicken were highly sensitive gentamycin and clostin. On the other hand, strains in the current study were highly resistant to doxycycline (100%) followed by florfenicol (85.7%)

and cefotaxime (57.1%). Such results agreed with those given by Hamed (1999). Furthermore, strains of *Staphylococcus* and *Streptococcus* were sensitive to amoxicilin/clavulanic acid, ciprofloxacine and gentamycine, while they were resistant to amoxicillin and sulphamethoxasole/trimethoprime (Adeleke and Omafuvbe, 2011). The bacterial colonization in epithelial surfaces is considered a critical first step in the pathogenesis of avian pathogenic *E. coli* isolates (Ramirez et al., 2009a). In the present study, the 20 serotypes isolates of *E.coli* were positive using the specific primers for Curli (Crl) gene and Fim H genes (adhesion encoding genes) using PCR. Such findings agreed with Khalil et al. (2012) who detected that results of amplification of adhesion encoding genes of *E. coli* isolated from cases with omphalitis using multiplex PCR (curli gene and fimbriae H gene) indicating that all pathogenic *E. coli* isolates tested for PCR gave positive.

**Table 11. Antibiogram test of *Staphylococcus* and *Streptococcus* spp. isolated from chicks with omphalitis**

| Antibacterial agents | *Staphylococcus* spp. | | | | | | *Streptococcus* spp. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | | Sensitive | | Intermediate | | Resistant | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Amoxicillin** | 0 | 0 | 0 | 0 | 5 | 100 | 0 | 0 | 0 | 0 | 2 | 100 |
| **Amoxicillin/clavulanic acid** | 3 | 60 | 0 | 0 | 2 | 40 | 1 | 50 | 0 | 0 | 1 | 50 |
| **Cefotaxime** | 2 | 40 | 2 | 40 | 1 | 20 | 0 | 0 | 0 | 0 | 2 | 100 |
| **Ciprofloxacin** | 4 | 80 | 0 | 0 | 1 | 20 | 2 | 100 | 0 | 0 | 0 | 0 |
| **Gentamicin** | 4 | 80 | 0 | 0 | 1 | 20 | 1 | 50 | 0 | 0 | 1 | 50 |
| **Sulphamethoxazole/ Trimethoprim** | 2 | 40 | 0 | 0 | 3 | 60 | 1 | 50 | 0 | 0 | 1 | 50 |

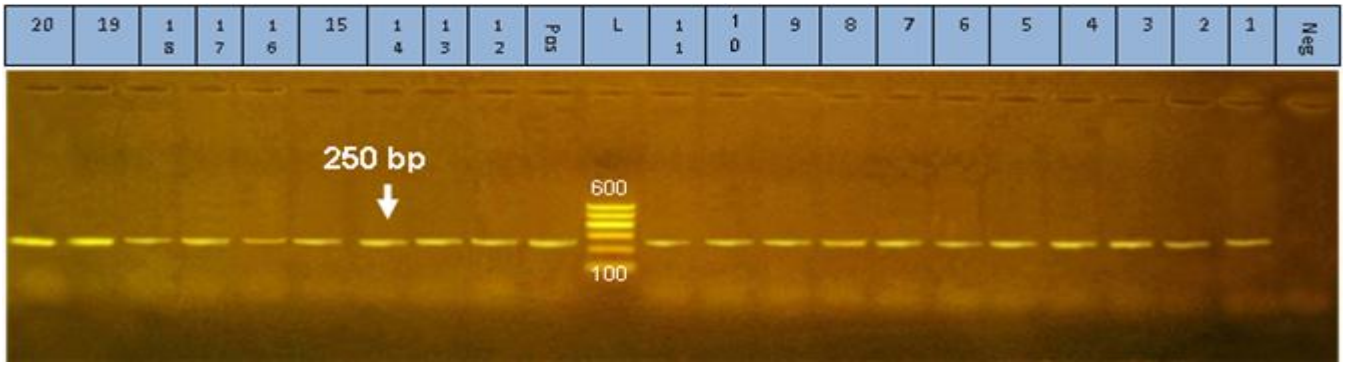


**Fig. 1. Electronic pattern of PCR products (250 bp) specific for Crl gene in agarose gel stained with ethid bromide.**

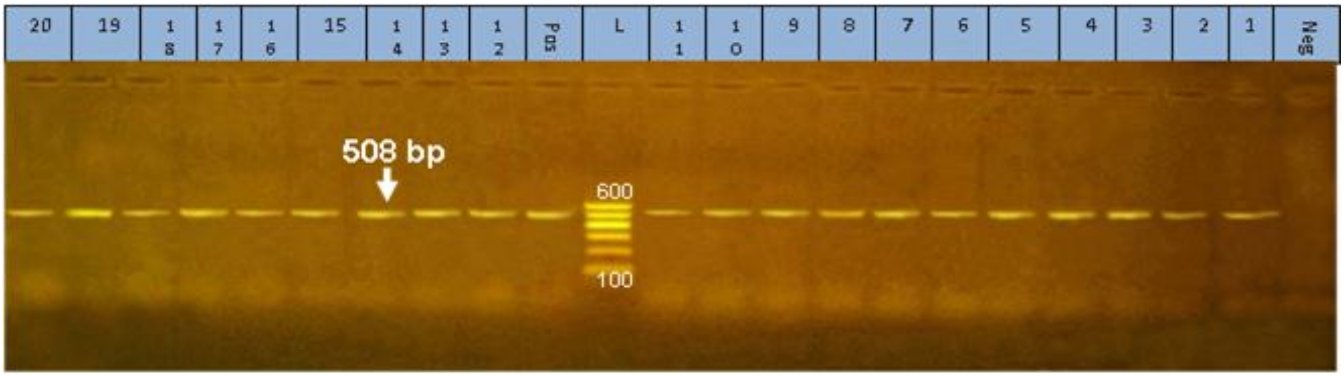


**Fig. 2. Electronic pattern of PCR products (508 bp) specific for FimH gene in agarose gel stained with ethid bromide.**

## 4. Conclusion

Chicks with omphalitis harbored various pathogens which could be considered a source of infection during the successive days of life in broiler chickens. Different serotypes of *E. coli* are present in omphalitis and all carry the adhesion encoding genes (crl and fimH genes). Serotypes of *Salmonella* were incorporated in such disease. The isolated pathogens showed variable a susceptibility to different antimicrobial drugs.

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