Prevalence of *E. Coli* in diseased chickens with its antibiogram pattern.

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**ABSTRACT**

Prevalence of *E. coli* among poultry farms was investigated in these studies, which cover different provinces in Egypt through examination of 400 chicken samples using standard methods for isolation and identification of *E. coli*. The result showed presence of *E. coli* among imported chicks in rate 44% and from local broiler chickens about 75%. The incidence of *E. coli* in on day old living diseased chicks was (58.3), while the incidence of *E. coli* from local broiler chickens was (71%) from living diseased chickens and about (83%) from freshly dead chickens These serotype of *E. coli* were O63, O103, O125, O158, O44. Antibiogram pattern test indicated the highest rate of resistant against tetracycline group where about 80% followed by the 2 β-Lactam antibiotic (73.3%), erythromycin about 63.3% of tested isolate were resistant, florfenicol about 53.3% were resistant, gentamycin was about 46.6% were resistance, finally ciprofloxacin about 40% of tested isolate were resistant by using disc diffusion method.

**Keywords:** *E. coli*, Diseased chickens, Antibiogram pattern.

1. INTRODUCTION

*E. coli* is a member of the family *Enterobacteriaceae*, which may constitute a great hazard to poultry industry causing high mortality, loss of weight and reduction of egg production (Bandyopadhay and Dhawedkar, 1984). *E. coli* infection is one of the serious problems that cause a great threat to the profitability of birds' enterprises all over the world. Although *E. coli* is a normal inhabitant of the intestinal tract of birds, under the influence of predisposing factors, like inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality, high mortality during rearing, reduced weight gain and condemnation of birds at the time of slaughter (Kaulet et al., 1992). Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with air sacculitis and associated pericarditis, perihepatitis and peritonitis being most typical (Ewers et al., 2003). The main clinical signs of naturally infected chicks with *E. coli* are reported as depression, loss of appetite, tendency to huddle respiratory distress, reduction of weight gain, drooped wing, closed eyes, cyanosis and labored breathing (Barnes, 1994). Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Cloud et al., 1985; Amara et al., 1995 and Blanco et al., 1998). This increasing resistance has received considerable national and international attention. The aim of the current work was establish to record the prevalence of *E. coli* infection in chickens
and the antibiogram pattern against the isolated strains.

2. MATERIAL AND METHODS

2.1. Chicken samples

A total of 400 chickens samples were collected from imported chicks and 100 from local broiler chickens. The samples were collected from different organ (liver, yolk sac, lungs and bone marrow) from different age (one day old –over one day old).

2.2. Detection of E.coli by conventional method: it was done according to Quinn et al. (2002)

2.2.1. Selective enrichment of E.coli

Each sample was inoculated separately into buffer peptone water and incubated at 37°C for 18 hrs under aerobic condition.

2.2.2. Colonization of E.coli on selective differential solid media

A loopful from the broth of each sample was streaked onto MacConkey's agar and Eosin Methylene blue agar. The inoculated plates were incubated at 37°C for 24 hours. Suspected E.coli colonies were purified and kept for further identification.

2.2.3. Identification of suspected E.coli colonies

It was performed according to Quinn et al., (2002): On MacConkey's agar and Eosin Methylene blue agar (EMB).

2.2.4. Microscopic examination

Gram's stain was prepared and used as described by (Cruickshank et al. 1975) for morphological study.

2.2.5. Biochemical Identification

According to Quinn et al. (2002) including Indole reaction, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test, Sugar fermentation test, Oxidase test, Triple sugar iron and Christener's urea agar test.

2.2.6. Serological identification of E.coli (Edwards and Ewing (1972)

Isolated strains were serotyped in animal health research institute, Dokki, Giza using: Polyvalent and monovalent diagnostic E.coli antisera.

2.3. Antibacterial sensitivity test

The disk diffusion technique was applied according to (Cruickshank et al., 1975). Seven type of antibiotics from different groups of antibiotics (ciprofloxacin, florfenicol, erythromycin, gentamycin, amoxicillin and ampicillin and tetracycline was used (The interpretation of inhibition zones of tested culture was according to NCCLS, 2002).

3. RESULTS

3.1. Incidence of E.coli infection in chicken

Examination of imported baby chicks revealed that detection of E.coli in 44% from 300 examined samples while in local broiler chickens revealed that detection of E.coli in 75% from 100 examined samples table (1).

Table (1) Incidence of E.coli recovered from examined chickens:

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of sample</th>
<th>No. of positive</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported chicks</td>
<td>300</td>
<td>133</td>
<td>44%</td>
</tr>
<tr>
<td>Local chickens*</td>
<td>100</td>
<td>75</td>
<td>75%</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>208</td>
<td>52%</td>
</tr>
</tbody>
</table>

* Broiler

225
Prevalence of *E. coli* in diseased chickens with its antibiogram pattern

### Table (2) Incidence of *E. coli* in different organs of one day old chick (imported chicks)

<table>
<thead>
<tr>
<th>Sample</th>
<th>No of examined</th>
<th>Different organs</th>
<th>Incidence of <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>liver</td>
<td>lung</td>
</tr>
<tr>
<td>Living diseased</td>
<td>180</td>
<td>50</td>
<td>27.7</td>
</tr>
<tr>
<td>Freshly dead</td>
<td>120</td>
<td>15</td>
<td>12.5</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>65</td>
<td>21.6</td>
</tr>
</tbody>
</table>

### 3.2. Incidence of *E. coli* in different organs of one day old chicks (imported chicks)

The internal organs of each chick were examined by bacteriological examination to determine the incidence of *E. coli* in each chick organ table (2). The incidence of *E. coli* in one day old living diseased chicks was (58.3%) while in freshly dead chicks it was (23.3%). The highest percentage of organ isolation was obtained from liver it was about 21.6%.

### Table (3) Incidence of *E. coli* in different organs of over one week old chickens (local broiler chickens):

<table>
<thead>
<tr>
<th>Sample</th>
<th>No of examined</th>
<th>Different organs</th>
<th>Incidence of <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>liver</td>
<td>lung</td>
</tr>
<tr>
<td>Living diseased</td>
<td>70</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Freshly dead</td>
<td>30</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

### 3.3. Incidence of *E. coli* in different organs of over one week old chicken (broiler chicken)

The incidence of *E. coli* in over one week old living diseased chickens was (71%) while in freshly dead chickens it was (83%). The highest percentage of organ isolation were obtained from bone marrow was about 30% from 100 sample (28%), table (3).

### 3.4. Serotyping of *E. coli* isolates recovered from chicken samples

The hundred and eight of *E. coli* strains recovered from different organs of chickens relieved that 158 strain can be identified serologically they belonged to different serogroups. The most commonly detected *E. coli* serogroups were O44, O158, O125, O103, O63, while 50 strain is not typed, table (4).

### 3.5. Antibiotic sensitivity of *E. coli* strains

The highest rate of resistance was shown against tetracycline group of antibiotic where about 80% of isolate were resistant followed by the 2 β-Lactam antibiotic (amoxicillin and ampicillin) (73.3%) followed by erythromycin about 63.3% of tested isolate were resistant, florfenicol about 53.3% were resistant, gentamycin is about 46.6% were resistance, finally ciprofloxacin about 40% of tested isolate were resistant (table 5).

### Table (4) Serotyping of *E. coli* isolates and its percentage

<table>
<thead>
<tr>
<th><em>E. coli</em> serotype</th>
<th>No of isolate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O44</td>
<td>18</td>
<td>11.3</td>
</tr>
<tr>
<td>O158</td>
<td>18</td>
<td>11.3</td>
</tr>
<tr>
<td>O114</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>O91</td>
<td>14</td>
<td>8.8</td>
</tr>
<tr>
<td>O125</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>O63</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>O55</td>
<td>1</td>
<td>3.6</td>
</tr>
<tr>
<td>O151</td>
<td>1</td>
<td>.3</td>
</tr>
<tr>
<td>O124</td>
<td>2</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Table (5) Result of antibiotics resistance of E.coli by disc diffusion method

<table>
<thead>
<tr>
<th>Isolate</th>
<th>G</th>
<th>E</th>
<th>T</th>
<th>C</th>
<th>A</th>
<th>M</th>
<th>FFC</th>
<th>AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>10</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Intermittent</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Resistance</td>
<td>14</td>
<td>19</td>
<td>2</td>
<td>1</td>
<td>22</td>
<td>22</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>% *</td>
<td>46</td>
<td>63</td>
<td>8</td>
<td>4</td>
<td>73</td>
<td>73</td>
<td>53</td>
<td>2</td>
</tr>
</tbody>
</table>


4. DISCUSSION

In the present study, E.coli was detected after pre-enrichment on BPW. Then inoculated direct on agar medium (MacConky agar, VRBL and EMB agar), Typical colonies on TSA were used for further morphological and biochemical identification (catalase, oxidase, indole, methyl red, vogasproskour, citrate, TSI, LI and urea) (Swayne et al., 1998). The typical E.coli colonies were typing by antisera. In the present work, all E.coli strains showed lactose fermentation (pink colonies) on MacConkey agar and/or VRBL agar medium and green metallic sheen colonies on EMB. Out of 400 chickens samples300 from imported chicks and 100 sample from local broiler chicken found 133 (44%) was positive for E.coli from imported chicks and 75(75%) positive E.coli from local broiler chickens. Table (1) (Zhao et al., 2001) demonstrated that the incidence of E. coli for imported chicks nearly similar to that observed in the present study. For example, the prevalence of E. coli in baby chicks was (38.7%). Interestingly, more recent investigations revealed much higher rates of E. coli for chicken (68%) obtained by Kegodeet al., 2008. The current results showed that E.coli isolates were recovered in the highest rate from chickens showing symptoms of colisepticaemia, indicating the role of the organism as potentially important avian pathogen. These finding agreed with those obtained by Khalid (1990); Mukhopadhyaya and Mishra (1992), Sripornomo et al., (1992) and Yun et al. (1997). E.coli was isolated from 52% of examined chicken almost similar percentages 47.3% in chickens were reported by Ramaswamyet al. (1982) and Barbour et al. (1985), who isolated E.coli from 40.4% of samples from colisepticaemia chickens. The incidence of E.coli in one day old living diseased chicks was (58.3%) while in freshly dead chicks it was (23.3%). The highest percentage of organ isolation was obtained from liver, it was about 21.6% from 300 isolate. The incidence of E.coli from local broiler chickens (over one week old) was 73% from living diseased chicken, while in freshly dead chicken it was (83%). The highest percentage of organ isolation was obtained from bone marrow was about 30% from 100 sample table (2). On another hand Tapan et al., (2012) detected colibacillosis from different farms. the highest isolation rate of E.coli from yolk sac (52.6%) and heart blood (38.4) in one day old -4week,and the highest percentage of E.coli isolation was from pericardial fluid (35.8%) followed by heart blood (33.4%) in older age (4-7 week).

AbdElatif (2004) examined 150 samples taken from five broiler chicken revealed the isolation of E.coli with percentage of 78.7%, where the isolation from apparently healthy chickens with percentage of 72.0% and clinically diseased chickens with percentage of 85.3%, respectively. From the isolated E.coli, 208 strains recovered from different organs of chickens relieved that 158 strains can be serotyped serologically. and belonged to different serogroups. The most commonly detected E.coli serogroups.
Prevalence of *E.Coli* in diseased chickens with its antibiogram pattern

were O44 (11.3%), O158 (11.3%), O125 (7.5%), O103 (9.4%), O63 (7.5%), O91 (8.8%), while 50 strain was un-typed. These results go hand to hand with the previous studies of Suwanichkul and Panigrahy (1988), Gross (1991) and Bosch et al. (1993), who reported that serogroups O44, O158, O114 and O91 were traditionally associated with colibacillosis in poultry (4). By using disc diffusion method showed that *E.coli* isolate were resistant to ciprofloxacin about 12 (40%), 14 (46.6%) of *E.coli* isolate resistance to gentamycin. Also 16 (63.3%) *E.coli* isolate were resistance to erythromycin antibiotic among 30 *E.coli* isolate. 16 (53.3%) showed resistance to florfenicol antibiotic among 30 isolate in these study (table5) nearly similar result were detected by Jiang et al., (2011) about 44.4% of isolate were resistant to ciprofloxacin a among chicken *E.coli* strain in China. On other hand Wang et al. (2001) found that high rates of resistance to quinolones have been reported from different parts of the world. In China, for example, more than 50% of the clinical strains of *E. coli* isolated during 1997-1999 were resistant to ciprofloxacin. Also Xia et al. (2009) observed that 198 avian *E.coli* isolates from Shandong, China were resistant to enrofloxacin, 2% resistance to gentamycin among fifty five *E.coli* isolate that disagree with our result while Makhol et al., (2011) demonstrated that (100%) of all tested isolates of *E.coli* strains isolated from poultry were resistant to erythromycin. Also Xinet al., (2007) observed that about 29% of *E.coli* isolate was resistance to florfenicol antibiotics in order to ensure the rational and effective use of these drugs. β-Lactam group (amoxicoline and ampicillin) showed 22(73.3%) resistant and that was similar agree with Guerra et al. (2003) who was found resistance in 40% *E.coli* strains isolated and multi resistance in 32% of the strains. The present study detected most of isolates was resistance to tetracycline with percent 80% by disc diffusion as shown in table (5) which agree with ‘Moon et al (2011) that studied the actual frequency of antimicrobial resistance in fecal Escherichia coli isolated from .One hundred and nine *E.coli* isolates were higher resistant to ampicillin (68.8%) streptomycin (60.6%), ciprofloxacin (65.1%), and tetracycline (96.3%). Chopra and Roberts, (2001) noticed the high rate of resistant to tetracycline was done due to inhibition bacterial protein synthesis by preventing attachment of t-RNA to ribosome.

**Conclusion:** Multidrug resistance among isolated *E.coli* which impair the poultry rearing and can affect the human health so antibiotics should be given after making sensitivity test to avoid misuse of drug as well as give in proper dose and recommended duration.

5. REFERENCES


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Prevalence of E. Coli in diseased chickens with its antibiogram pattern

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