Isolation and characterization of avian pathogenic *Escherichia coli* from broiler chickens in some Governorates of Egypt

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Abstract

A total of 80 samples (liver, heart, lung and gall bladder) were collected from diseased and freshly dead broiler chickens from different poultry farms reared in (Zagazig) Sharkia, Ismailaia, Sinai, (Dahshor) Giza and Kaluobaia Governorates for bacteriological examination for *E*-coli infection. The results revealed that 49 out of 80 samples (61%) were positive for *E*-coli isolation. The result of in vitro Pathogenicity testing by Congo red (CR) binding assay indicates that 14 isolates (28.6 %) were CR positive. The most predominant serotypes were O125,O114 and O44 as each represent (2) isolates out of (14) followed by O78, O86, O158, O127, O91, O25, O119 and untypable as each represent (1) isolate out of (14) isolates. Now in this study we are straining and updating available circulating strains of avian pathogenic *Escherichia coli* (APEC) micro-organisms to control colibacillosis in broiler chickens either by treatment or vaccination.

Key Words: Pathogenic -*E*-coli – Broiler – Isolation – Congo red assay.

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Introduction

*Escherichia Coli* strains designated as avian pathogenic *E*-Coli (APEC) are responsible for avian Colibacillosis, acute and largely systemic disease that promotes significant economic losses in poultry industry worldwide because of mortality increase, medication costs, and condemnation of carcass. APEC is a subgroup of extra-intestinal pathogenic *E*-Coli pathotype, which includes uropathogenic *E*-Coli, neonatal meningitis *E*-Coli and septicemic *E*-Coli (Kobayashi et al., 2011). Several reports are available on the involvement of serotypes of *E*-coli in poultry diseases (Roy et al., 2004) and so on. The pathogenic and non-pathogenic strains in poultry are differentiated based on the virulence which has been attributed to various factors like fimbriae, production of colicin, motility and embryo lethality. Hence, detection of these strains become important for effective treatment and control (Susantha et al., 2001). In view of the significance of *E. coli* infection in poultry.

This study has been undertaken to isolate and characterize *E*-coli form different pathological conditions and to determine the prevalent serotypes and there in vitro pathogenicity.

Material and Methods

2.1. Sample collection & Transportation:

A total of 80 samples (liver, heart, lung and gall bladder) were collected from diseased freshly dead broilers chickens from 80 different poultry farms located in Sharkia, Ismailaia, Sinai, Giza and Kaluubaia Governorates in separate zipper lock bag, kept in ice box and immediately transported to the laboratory. Dead chickens showed lesions of Colibacillosis or chronic respiratory disease (CRD). The available flocks history with their relation to the previous work researches are shown in table (3).

2.2. Bacteriological examination:
A loopfuls from each were organs inoculated onto nutrient broth and incubated aerobically at 37ºC for 12 hours. Loopfuls from incubated nutrient broth were streaked onto Eosin methylene blue (EMB) agar plates and incubated for 24 hours at 37ºC. Suspected colony was picked up and streaked on the MacConkey’s agar plates then incubated for another 24-48 hours at 37ºC. The suspected lactose fermented colonies were picked up and kept in Semi-solid agar for morphological and biochemical identification (Konemann et al., 1992; Quinn et al., 2002).

2.3. In Vitro Pathogenicity Testing:
Various serotypes were tested for pathogenicity based on Congo red dye binding test as per the technique of Berkhoff and Vinal (1986). Each isolate was cultured on a separate plate of Trypticase soy agar supplemented with 0.003% Congo red dye (Sigma) and 0.15% bile salts. Appearance of deep brick red colonies after incubated at 37°C for 24 hrs was recorded as positive.

2.4. Serological typing of E-coli:
The obtained 14 biochemically and Congo red positive E-coli isolates were subjected to serological identification (Edward et al., 1972) using slide agglutination test.

Results
On Eosin Methylene blue (EMB) agar showed distinctive green metallic sheen with a black center colonies, medium sized rounded pink colonies on MacConkey’s agar media while showed small sized dark brick red colonies on Congo red dye media as a result of in vitro pathogenicity test. E-coli isolates were Gram negative, motile, non-sporulated, medium sized bacilli arranged single, pairs and in groups when stained with Gram’s stain. The suspected E-coli isolates were confirmed by biochemical tests and showed negative Urease test, negative Citrat utilization test, positive Indole test, (A/A with H2S gas production and A/A without H2S gas production) Triple sugar(TSI), (+ve) Motility on semisolid agar media. There were found inactive biotypes that were an aerogenic.

It was found that 2 serotype was isolated from the same bird; serotype (O114) and serotype (O158); suspected colibacillosis diseased freshly dead broiler chicken (samples no. 5 and 6). The most predominant serotypes were O125, O114 and O44 as each represent two isolates out of fourteen followed by O78, O86, O158, O127, O91, O25, O119 and untypale as each represent one isolates out of fourteen. There was found that we could isolate serotype (O125) and serotype (O44) from liver of sample no. (8) & heart of sample no. (7) of suspected colibacillosis diseased dead native breed broilers of the same flock. The results of the Congo red (CR) binding assay indicates that 14 isolates (28.6 %) were positive and 35 isolates (71.4%) were negative.

Table (1): Serogrouping of biochemically positive E-coli isolated from broilers chickens:

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Somatic Antigen</th>
<th>Capsular Antigen</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O125</td>
<td>K70</td>
<td>O125(K70)</td>
</tr>
<tr>
<td>2</td>
<td>O119</td>
<td>K69</td>
<td>O119(K69)</td>
</tr>
<tr>
<td>3</td>
<td>O78</td>
<td>K80</td>
<td>O78(K80)</td>
</tr>
<tr>
<td>4</td>
<td>O86</td>
<td>K-</td>
<td>O86(K-)</td>
</tr>
<tr>
<td>5</td>
<td>O114</td>
<td>K90</td>
<td>O114(K90)</td>
</tr>
<tr>
<td>6</td>
<td>O158</td>
<td>K-</td>
<td>O158(K-)</td>
</tr>
<tr>
<td>7</td>
<td>O125</td>
<td>K70</td>
<td>O125(K70)</td>
</tr>
<tr>
<td>8</td>
<td>O44</td>
<td>K74</td>
<td>O44(K74)</td>
</tr>
<tr>
<td>9</td>
<td>O127</td>
<td>K63</td>
<td>O127(K63)</td>
</tr>
<tr>
<td>10</td>
<td>O114</td>
<td>K90</td>
<td>O114(K90)</td>
</tr>
<tr>
<td>11</td>
<td>O91</td>
<td>K-</td>
<td>O91(K-)</td>
</tr>
<tr>
<td>12</td>
<td>O44</td>
<td>K74</td>
<td>O44(K74)</td>
</tr>
<tr>
<td>13</td>
<td>O25</td>
<td>K11</td>
<td>O25(K11)</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>Untypale</td>
</tr>
</tbody>
</table>

Table (2): Distribution of Congo red positive E-coli Serotypes isolates in relation to tissues of broiler chickens
Table (3): The relationship between the isolated serotypes from field case and the previous work researches:

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>O type &amp; organ</th>
<th>Breed</th>
<th>Age / history</th>
<th>PM lesions</th>
<th>Previous Work Researches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O125 liver</td>
<td>White broilers</td>
<td>32 days, 8,000, 60 dead daily</td>
<td>perihepatitis, pericarditis and airsacculitis.</td>
<td>Andrawis(1980), Zahdeh(1982), Sahar Zou El-Fakar (1994).</td>
</tr>
<tr>
<td>6</td>
<td>O158 gall bladder</td>
<td>Sasso</td>
<td>56 days, 15 dead daily</td>
<td>CRD</td>
<td>Mukherjee et al.,(1997), Shahin et al., (2011), Jana et al.,(2013)</td>
</tr>
<tr>
<td>7</td>
<td>O125 liver</td>
<td>native breed</td>
<td>42 days, 10,000, 25 dead daily</td>
<td>CCRD</td>
<td>Andrawis (1980), Zahdeh (1982), Sahar Zou El-Fakar (1994).</td>
</tr>
<tr>
<td>8</td>
<td>O44 heart</td>
<td>native breed</td>
<td>42 days, 10,000, 25 dead daily</td>
<td>CCRD</td>
<td>Nagwa (1995), Shahin et al. (2011)</td>
</tr>
<tr>
<td>11</td>
<td>O25 liver</td>
<td>White broiler</td>
<td>16 days, 8,000, 8 dead daily</td>
<td>CRD</td>
<td>Sinha et al (1985), Musumeci et al(2012), Jana et al.,(2013)</td>
</tr>
<tr>
<td>13</td>
<td>O44 liver</td>
<td>White broiler</td>
<td>25 days, 6,000, 30 dead daily</td>
<td>CRD</td>
<td>Nagwab(1995), Shahin et al.(2011)</td>
</tr>
<tr>
<td>14</td>
<td>Untypable heart</td>
<td>Sasso</td>
<td>56 days, 15 dead daily</td>
<td>CRD</td>
<td>Taha et al.,(2002), Shahin et al.(2011)</td>
</tr>
</tbody>
</table>
Discussion

*Escherichia coli* infections in birds cause many clinical manifestations which characterized by a respiratory disease that is frequently followed by a generalized infection which end by death. Avian pathogenic *E. coli* (APEC) strains fall under the category of extra intestinal pathogenic *E. coli*, which are characterized by the possession of virulence factors that enable to live extra intestinal life (Johnson *et al.*, 2006).

The serotypes isolated in this study were in accordance with Ibrahim *et al.* (1998) and Singh and Gupta (1996). The very low per cent Serotypes O2 and O78 may probably be due to variation in serotypes over a period of time in a particular area (Belitski and Panika, 1969). The correlation between the isolates and the disease condition could not be established (Mukherjee and Mishra, 1995).

The results of *in vitro* pathogenicity testing were in agreement with Berkhoff and Vinal (1986), who also reported a strong correlation between expression of CR phenotype and virulence in avian *E. coli* and suggested that it was associated with the presence of p-D-glucan in bacterial cell wall. Previous studies also indicated that isolates of virulent avian *E. coli* can be identified by their ability to bind Congo red (Singh and Gupta, 1996). The characteristic of CR binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker. Nevertheless, Yoder (1989) has reported that Congo red binding did not correlate well with pathogenicity.

In conclusion, the present study clearly demonstrates that *E. coli* is one of the major pathogen responsible for various types of disease conditions in poultry leading to economic losses to poultry industry. Almost serotypes of *E. coli* isolated have been found to be pathogenic, but no particular serotype could be attributed to a particular disease condition or a particular age group.

References


Gomis, S. M.; Riddell, C.; Potter, A. A. and Allan, B. J. (2001): Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with


