ANTIBIOTIC SUSCEPTIBILITY, SEROTYPING AND PATHOGENICITY DETERMINATION OF AVIAN ESCHERICHIA COLI ISOLATED FROM COLIBACILLOSIS CASES IN BROILER CHICKEN IN ALJABEL ALAKHDAR REGION, LIBYA

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ABSTRACT

The main serotypes, pathogenicity, and antibiotic susceptibility of Avian Escherichia coli isolated from Colibacillosis cases in the Aljabel Al Akhdar region of Libya were investigated in this study. To determine the pathogenicity of E.coli isolates, Congo red binding, hemolysis, and lethality tests were conducted on Seventy-two unvaccinated broilers chicks on the day of hatching. Avian pathogen serotypes and antibacterial susceptibility testing to twelve antibiotics were also performed according to the standard methods. Results revealed that the only (O114) serotype that had been orally inoculated caused mortality on the first day. While all E.coli isolates inoculated by subcutaneous route showed mortality on the first day. The highly pathogenic serotypes were (O26) induced (100%) mortality. Congo red (CR) binding assay showed that (100%) of isolates were positive. Only (O26) was found to be hemolytic. Serotyping results revealed that E.Coli strains belong to serotypes (O26, O111, O114, O119). According to the results, (100%) of E.Coli isolates were less sensitive to Cephalothin, Lincomycin, as well as Erythromycin, while the sensitivity level to Amoxyclov and Doxycycline was (40%) and (60%) respectively. Only (20%) of E.Coli isolates showed resistance to Enrofloxacin, Neomycin, Streptomycin, and Tetracycline. All strains were susceptible to Imipenem (100%). Significantly, O26 serotype was highly resistant (75%) to 9 out of 12 different antibiotics tested.

Keyword: Pathogenicity; Serotyping; Susceptibility; E.Coli

INTRODUCTION

It has been found the focus of several international type of research has intensified their studies on Escherichia Coli due to its involvement in a wide outbreak of gastrointestinal illness in animals and humans (Deshmukh and Karpe, 2006). A link between E.Coli and human illness has been discovered since chicken has been extensively embraced as a conveniently available source of meat. In Poultry, Colibacillosis is the most frequently reported infection during the year (Jones et al., 2000, Chowdhury and Das, 2003). It
has been established as a common death causes in domestic birds (Kumar et al., 2005).

Colibacillosis is the most dominant bacterial disease in Chickens or Turkeys. It can cause a variety of symptoms of all ages, such as yolk sac omphalitis syndromes during the first week of life when the transmission of E.Coli occurs by contaminated eggs or it is caused by inadequate induced hatchery sanitation. Colibacillosis commonly occurs as a secondary infection and can cause respiratory infections leading to pericarditis, perihepatitis and/or airsacculitis. E.coli can also cause synovitis and osteomyelitis as a result of systemic infection. Some types of E.Coli are only pathogenic to Chicken [APEC, Avian pathogenic E.Coli]. These strains are O1, O2, O35 and O78. Colicin and type1 fimbrial seem to be correlated with virulence; however non-APEC strains can sometimes also cause Colibacillosis. Many E.Coli strains which cause septicemia are associated with O Serogroups: O1, O2, O35, and O78, according to several surveys (Gross, 1958; Sojka and Carnaghan, 1961; Cheville and Arp, 1978; Cloud et al., 1985; Whittam and Wilson, 1988 and Dozois et al., 1992). However, other researchers found that avian Colibacillosis isolates have a large antigenic diversity (Allan et al., 1993 and White et al., 1990, 1993).

The extent to which a specific O serogroup is involved in illness is assumed to be dependent on geographically dependent. Although E. Coli can be easily cultured, further serotyping is needed to identify whether E.Coli isolates belong to APEC or not (Ulrich et al., 2008). Antimicrobial susceptibility testing of animal infections in vitro can assist veterinarians in selecting the most appropriate treatment (Jesus et al., 1997). Strains of resistant E.Coli strains identified from cases suffering from colibacillosis are becoming more common, according to in vitro antibiotic sensitivity testing. Trimethoprim Sulphamethoxazole (67%) and the new fluoroquinolones were resistant with a percentage of (13 to 24 %) (Cloud et al., 1985; Goren, 1990; Allan et al., 1993; Amara et al., 1995; Peighambari et al., 1995 and Jesus et al., 1997). Either pathogenic or non-pathogenic organisms are valuable indicators when research is dependent on investigating the virulence characteristics of bacteria (Berkhoff and Vinal, 1986). The current research was carried out on the E.Coli isolate from broiler farms in Al Jabal Al Akhdar region to identify serogrouping and pathogenicity, as well as to determine the efficacy kinds of antibiotics commonly used, the virulence of E.Coli was also investigated.

MATERIALS AND METHODS

Bacterial isolates:
Five of E.Coli strains, recovered from diseased broiler, were obtained from the preventive medicine and public health laboratory, faculty of veterinary medicine, Omar AL-Mukhtar University, Libya.

Serotyping:
The identification of (O) somatic antigens was undertaken at the animal health research centre, Egypt.

Antimicrobial susceptibility test:
According to the approach performed by (Bauer et al., 1966), all E.Coli were evaluated by using the disk diffusion method on Muller Hinton agar (HiMedia). Antibiotics discs of Oxoid used in this work were Amoxyclyav (AC) (10μg),Imipenem (IMP) (10 μg), Streptomycin (S) Colistin sulphate (CT) (25μg), Cephalothin (KF) (30 μg), Doxycycline (DO) (30μg), Tetracycline (TE) (30μg) Erythromycin (E) (15μg), Enrofloxacin (ENR) (5μg), Gentamycin (GEN) (10μg), Lincomycin (Lin) (15 μg), Neomycin (N) (30μg), (10μg).
**In vitro pathogenicity test:**

It was evaluated by:

**A- Hemolysis test:**

A plate of blood agar base containing 5% sheep blood was streaked with an overnight culture of *E. coli* isolates. Then it was incubated at 37°C for 24 hrs, and examined for a clear zone of hemolysis, indicating that it was hemolysis positive

**B- Congo Red dye- binding test:**

*E.Coli* isolates were streaked on Tryptic soy agar containing an addition of 0.03% Congo red and 0.15% bile salt and incubated for 24 hours at 37°C. Congo red positive was defined as the existence of red colonies, whilst white or grey colonies were classified as negative.

**In vivo pathogenicity test:**

A total of Seventy-two one-day-old unvaccinated broiler chicks (Breed: F Is hybrid) of different sex selected on the first day of life from an industrial hatchery (Kandula Company). Chicks were fed ad libitum during the experiment. Chicks were distributed into a completely randomized design to five groups with six birds each. The 5th group was left as a control. Five *E.Coli* strains including O26, O111, O114, and O119 were used; therefore, four groups were inoculated either subcutaneously or orally with a single dose of 0.25 ml of brain-heart broth per chick, containing an average of 3x10^6 CFU of each isolate.

Birds were maintained for seven days post-inoculation and daily monitored for mortality. Dead birds were examined for lesions of septicemia (fibrinous perihepatitis, pericarditis, airsacculitis and Synovitis). On the seventh days of age, the survival birds (Sick and healthy) were sacrificed and subjected to post mortem examination. Reisolates of *E. Coli* from the liver and organs were done on MacConky, tertitol 7 agar, and cong red (CR) agar. *E.Coli* strains that produced any or all of the above lesions were considered invasive (Capable of producing septicemic disease).

**RESULTS**

**Serological Identification:**

*E.coli* isolates were serologically identified to different O groups. The most commonly isolated subgroups were O26, O111, O114, and O119 as shown in Table (1).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC6</td>
<td>O111</td>
</tr>
<tr>
<td>EC11</td>
<td>O26</td>
</tr>
<tr>
<td>EC12</td>
<td>O119</td>
</tr>
<tr>
<td>EC13</td>
<td>O114</td>
</tr>
<tr>
<td>EC10</td>
<td>O26</td>
</tr>
</tbody>
</table>

**Antibiotic susceptibility of E.Coli isolates:**

The antimicrobial susceptibility of *E.Coli* subtypes outlined different percentages of resistance and sensitivity to the used antibiotics as utilized in this investigation Table (2). (100%) of *E.Coli* isolates were recorded to be resistant to Cephalothin, Lincomycin, and Erythromycin, while relatively few isolates were resistant (40%, 60%) to Amoxyclav and Doxycycline, respectively. Only (20%) of *E.Coli* isolates exhibited resistance to Enrofloxacin, Neomycin, Streptomycin and Tetracycline. All *E.Coli* isolates have a high level of sensitive (100%) to Imipenem. Significantly, O26 serotype was highly resistant (75%) to 9 out of 12 different antibiotics tested.
Table 2: Antibiotics susceptibility profile of E.Coli strains.

<table>
<thead>
<tr>
<th>E.Coli strains Serotypes</th>
<th>Antibiotics</th>
<th>Strain resistant percentages %</th>
<th>Strain sensitivity percentages %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC6 O_{111}</td>
<td>IMP M DO M CT M KF M Lin M GEN M E M AC ENR M N M S M TE</td>
<td>25%</td>
<td>16</td>
</tr>
<tr>
<td>EC10 O_{26}</td>
<td>IMP M DO M CT M KF M Lin M GEN M E M AC ENR M N M S M TE</td>
<td>41%</td>
<td>8.3</td>
</tr>
<tr>
<td>EC11 O_{26}</td>
<td>IMP M DO M CT M KF M Lin M GEN M E M AC ENR M N M S M TE</td>
<td>75%</td>
<td>8.3</td>
</tr>
<tr>
<td>EC12 O_{119}</td>
<td>IMP M DO M CT M KF M Lin M GEN M E M AC ENR M N M S M TE</td>
<td>25%</td>
<td>50</td>
</tr>
<tr>
<td>EC13 O_{114}</td>
<td>IMP M DO M CT M KF M Lin M GEN M E M AC ENR M N M S M TE</td>
<td>33%</td>
<td>8.3</td>
</tr>
</tbody>
</table>

IMP=Imipenem, DO=Doxycycline, CT=Colistin sulphate, KF=Cephalothin, Lin=Lincomycin, GEN=Gentamycin, E=Erythromycin, AC=Amoxyclav, ENR=Enrofloxacin, N=Neomycin, S=Streptomycin, TE=Tetracycline.

S=Sensitivity= m≥2.5mm anta bet 30ne
R=Resistant= m<0.0mm anta bet 30ne
M=Moderate= m1.2mm anta bet 30ne

Results showed that only two chicks died (33%) during the first-day post inoculation by oral route in O_{114} serotype group Table (3), while all E.Coli inoculated subcutaneously showed mortality on the first day Table (4). The mortality rate was (100%) for O_{26} within 24-48 hours post-inoculation. The Control group all survived.

Table 3: Showing the mortality rate after 1 and 7 days post inoculation by oral and route of E.Coli strain.

<table>
<thead>
<tr>
<th>E.Coli strain</th>
<th>Serotype</th>
<th>A mortality rate after 1 day post inoculation by oral route</th>
<th>mortality rate after 7 days post inoculation by oral route</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC6</td>
<td>O_{111}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EC11</td>
<td>O_{26}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EC12</td>
<td>O_{119}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EC13</td>
<td>O_{114}</td>
<td>2 (33%)</td>
<td>-</td>
</tr>
<tr>
<td>EC10</td>
<td>O_{26}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cont-ve</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

These data collected during a separate experiment
Con-ve = control negative group.
-no death
Table 4: Showing the mortality rate after 1 and 7 days post inoculation by and subcutaneous route of E.Coli strain.

<table>
<thead>
<tr>
<th>E.Coli strain</th>
<th>Serotype</th>
<th>A mortality rate after 1 days post inoculation by subcutaneous route</th>
<th>mortality rate after 7 days post inoculation by subcutaneous route</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC6</td>
<td>O111</td>
<td>2(33%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>EC11</td>
<td>O26</td>
<td>6(100%)</td>
<td>-</td>
</tr>
<tr>
<td>EC12</td>
<td>O119</td>
<td>2(33%)</td>
<td>4 (60%)</td>
</tr>
<tr>
<td>EC13</td>
<td>O114</td>
<td>2 (33%)</td>
<td>4 (60%)</td>
</tr>
<tr>
<td>EC10</td>
<td>O26</td>
<td>6(100%)</td>
<td>-</td>
</tr>
<tr>
<td>Cont-ve</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

These data collected during a separate experiment
Con-ve = control negative group.
-no death because all group members died after 1 day post inoculation

Post mortem lesions in most carcasses showed severe to a mild degree of omphalitis (watery and congested yolk sac), hydro pericardium, visceral congestion, enlargement and congestion of liver, pericarditis, and precipitated urea in the ureter, congested lung, and mild airsaculitis. These lesions were displayed in chicks that died during the first two days post inoculation. No signs and lesions were reported in the control group.

Hemolysis test:
Results showed that only one isolate O26 produced hemolysis on sheep blood agar.

Congo red test:
Result showed that all E.Coli isolates (100%) were positive to (CR).

DISCUSSION

Serological identification of E.Coli isolates that used in this work were belonged to the O26, O111, O114, and O119 Table (1). All of these Serogroups are belong to the Enteropathogenic E.Coli (EPEC), a phrase defined by (Neter, 1959) to characterize the serogroups of E.Coli, epidemiologically relevant to the epidemic of diarrhea in infants in the 1940s and the 1950s in the UK. As previously noted by (Ashraf et al., 2015), O111 serotype was recovered from a newly hatched chicken. (El-Jakee et al., 2012) obtained O26 and O111 from chicken cloacal swabs. Another study by (Schroeder et al., 2002) identified O26 and O111 from humans, cows, turkey, and chicken.

The evaluation of the pathogenic bacterial susceptibility profile is a significant therapy guide to decrease the economic loss of E.Coli infection (Costa et al., 2010). In this study, a resistance towards antimicrobial in the class of Cephalosporins, and Penicillins were observed. These findings were in accordance with the previous study considering the usage of these antimicrobial as a crucial contributor in the development of antimicrobial resistance E.Coli (Schroeder et al., 2002). All of these isolates showed resistance for Cephalothin, Lincomycin, and Erythromycin Table (2). Nevertheless, they were highly sensitive to Imipenem. This was in agreement with (Khan et al., 2014). This can be due to the uncommon use of Imipenem in the poultry industry in this country. In the current study, a moderate sensitivity to Enrofloxacin was reported, this was in contrast to the study by (Robert et al., 2002), who showed resistance to Enrofloxacin (28%). (Jesus et al., 1997) observed a high level of resistance (94%) to Tetracycline, whereas (20%) of resistance level was obtained in this study.
Indiscriminate use of antibiotics and the rise of Carbapenem-resistant bacteria, according to a recent world and health organization report, are an alarming worldwide health hazard (WHO, 2014). As a result, the emergence and spread of carbapenem-resistant E.Coli strains can pose a threat to global public health. (Wen et al., 2018). E.Coli is one of the most important etiological agents both for nosocomial and community –acquired infection in humans and the possibility of the transmission of resistant bacteria from meat-producing animals has been reported (Petersen et al., 2002; Oteo et al., 2005).

As mentioned in table (3), only one serotype (O_114) strain caused the death at first- day post- inoculation by oral route, whereas by subcutaneous inoculation all E.Coli strains caused death ranging from 60% to 100% table (4). These findings were in contrast with a study by (Goren, 1978) showed that chicks were much more susceptible to intra tracheal than oral inoculation. Also, he mentioned that serotype O_{78} appears to be much more pathogenic than O_{26}. In our study, O_{26} was more pathogenic than other studied serotypes. Furthermore, septicemia was the most frequent challenge that occurred in either died or authorized birds subcutaneously inoculated. Accordingly, two out of five serotypes (O_{26}, O_{114}) were classified as highly virulent serotypes.

A direct correlation had been found between Congo red binding and virulence for some bacteria and their ability to cause septicemia (Ellakany et al., 2019). All tested isolates of different serogroups were Cong Red positive. (Berkhoff and Vinal, 1986) found that clinically isolated E.Coli, which were (CR+), caused septicemic infection in chickens.

Hemolytic activity on blood agar is a characteristic of certain strains of E.Coli (Melese, 2015). It was mentioned that hemolysin is considered an important mark of Entero-toxigenic virulence E.Coli. In the current study, one out of five different serotypes (O_{26}) expressed hemolysin on sheep blood agar. On the contrary, (Brenda et al., 1993) reported that O_{1}, O_{2}, and O_{78} were the predominant serogroups involved in Colibacillosis, except that all of these serogroups did not produce hemolysin on sheep blood agar.

CONCLUSION

Multiresistance E.Coli strains have emerged as a serious worldwide problem. As a result, rising antibiotic resistance knowledge and encouraging sensible antibiotic usage is critical to fighting infectious illnesses that affect human and animal health. It is not enough to minimize antibiotic use in terms of quantity; rather, it is necessary to enhance antibiotic usage in terms of quality.

REFERENCES


microbiology, immunology and veterinary Public health.


