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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 (O_{114}) 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 (O_{26}) induced (100%) mortality. Congo red (CR) binding assay showed that (100%) of isolates were positive. Only (O_{26}) was found to be hemolytic. Serotyping results revealed that *E. coli* strains belong to serotypes (O_{26}, O_{111} $O_{114,}$ O_{119}). According to the results, (100%) of *E. coli* isolates were less sensitive to Cephalothin, Lincomycin, as well as Erythromycin, while the sensitivity level to Amoxyclav 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, O_{26} 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

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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 airsac-culitis. *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 O_1, O_2, O_{35} and O_{78}. 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 (B erkhoff 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 center, 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 Amoxyclav (AC) (10 µg), Imipenem (IMP) (10 µg), Streptomycin (S) Colistin sulphate (CT) (25 µg), Cephalothin (KF) (30 µg), Doxycycline (DO) (30 µg), Tetracycline (TE) (30 µg) Erythromycin (E) (15 µg), Enrofloxacin (ENR) (5 µg), Gentamycin (GEN) (10 µg), Lincomycin (Lin) (15 µg), Neomycin (N) (30 µg), (10 µg).

***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 37C° 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 O_{26}, O_{111}, $O_{114,}$ and O_{119} 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 seven 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, tergitol 7 agar, and congo 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 O_{26}, O_{111}, $O_{114,}$ and O_{119} as shown in Table (1).

Table 1: Presenting (O) serotypes identified from avian *E. coli* isolates.

Strain	Serotype
EC_6	O_{111}
EC_{11}	O_{26}
EC_{12}	O_{119}
EC_{13}	O_{114}
EC_{10}	O_{26}

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, O_{26} serotype was highly resistant (75%) to 9 out of 12 different antibiotics tested.

Table 2: Antibiotics susceptibility profile of *E. coli* strains.

E. coli strains	Serotypes	Antibiotics												Strain resistant percentages %	Strain sensitivity percentages %
		IMP	DO	CT	KF	Lin	GEN	E	AC	ENR	N	S	TE		
EC6	O_{111}	S	M	M	R	R	M	R	S	M	M	M	M	25%	16
EC10	O_{26}	S	R	M	R	R	M	R	R	M	M	M	M	41%	8.3
EC11	O_{26}	S	R	M	R	R	M	R	R	R	R	R	R	75%	8.3
EC12	O_{119}	S	M	M	R	R	M	R	S	S	S	S	S	25%	50
EC13	O_{114}	S	R	M	R	R	M	R	M	M	M	M	M	33%	8.3

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= ḿ2.5mm anta bet 30ne
R=Resistant= ḿ0.0mm anta bet 30ne
M=Moderate= ḿ1.2mm 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.

E. coli strain	Serotype	A mortality rate after 1day post inoculation by oral route	Mortality rate after 7 days post inoculation by oral route
EC6	O_{111}	0	0
EC11	O_{26}	0	0
EC12	O_{119}	0	0
EC13	O_{114}	2 (33%)	-
EC10	O_{26}	0	0
Cont-ve	-	0	0

These data collected during a separate experiment
Con-ve = control negative group.
-no death

Table 4: Showing the mortality rate after1 and 7 days post inoculation by and subcutaneous route of *E. coli* strain.

E. coli strain	Serotype	Mortality rate after 1 days post inoculation by subcutaneous route	Mortality rate after 7 days post inoculation by subcutaneous route
EC6	O_{111}	2(33%)	4 (100%)
EC11	O_{26}	6(100%)	-
EC12	O_{119}	2(33%)	4 (60%)
EC13	O_{114}	2 (33%)	4 (60%)
EC10	O_{26}	6(100%)	-
Cont-ve	-	0	0

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 (O_{26}) 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 O_{26}. O_{111}, $O_{114,}$and O_{119} 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), O_{111} serotype was recovered from a newly hatched chicken. (El-Jakee *et al.*, 2012) obtained O_{26} and O_{111} from chicken cloacal swabs. Another study by (Schroeder *et al.,* 2002) identified O_{26} and O_{111} 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 Ertyhromycin 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 Enroro-floxacin 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

Allan, B.J.; Van den Hurk, J.V. and Potter, A.A. (1993): Characterization of *Escherichia Coli* isolated from cases of avian colibacillosis. Can. J. Vet. Res. 57, 146-151.

Amara, A.; Ziani, Z. and Bouzoubaa, K. (1995): Antibioresistance of *Escherichia Coli* Strains isolated in morocco from chickens with Colibacillosis. Vet. Microbiol. 43(4): 325-330.

Ashraf A. Abd El Tawab; Ahmed M. Ammar; Soad A. Nasef and Reem M. Reda (2015): Antibacterial resistance and resistance gene detriments of *E. coli* isolated from chicken. Benha veterinary medical journal, 28(2): 231-240.

Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Truck, M. (1966): Antibiotic susceptibility testing by standardized single disk method. Amer. J. Clin. Pathol., 45: 493-496.

Berkhoff, H.A. and Viral, S.C. (1986): Congo red medium to distinguish between invasine and Non- Invasive

Escherichia Coli pathogenic for poultry. Avian Dis., 30(1):117-121.

Brenda, Y.; Allan, J.V.; Hurk, V.D. and Adrew, A.P. (1993): Characterization of *Escherichia Coli* isolation for cases of Avian Colibacillosis. Can. J. Vet. Res. (57): 146-151.

Chowdhury, M. and Das, R. (2003): Incidence of drug resistance in *E. coli* strains in West Bengal. Ind. Vet. J. 80:81-82.

Cheville, N.F. and Arp, L.H. (1978): Comparative pathogenic finding of *Escherichia Coli* infection in birds. JAVMA 173(5 pt 2): 584-587.

Cloud, S.S.; Rosenberger, J.K.; Fries, P.A.; Wilson, R.A. and odor, E.M. (1985): In vitro and vivo characterization of avian *Escherichia Coli*, I, serotypes, metabolic activity, and antibiotic sensitivity. Avian Dis. 29(4), 1084-1093.

Costa, M.M.; Drescher, G.M.; Aboni, F.; Weber, S.S.; Schrank, A.; Vainstein, M.H.; Schrank, I.S. and Vargas, A.C. (2010): Virulence factors, antimicrobial resistance, and plasmid content of *Escherichia Coli* isolated in Swine commercial farms. Arq. Bras. Med. Vet. Zootec.62 (1): 30-36.

Deshmukh, A.S. and Karpe, A.G. (2006): In vitro transfer of plasmid in *E. coli* .Ind. J. Comp. Microbial. Immunol. Infect. Dis. (27): 32-34.

Dozois, C.M.; Fairbrother, J.M.; Hasel, J. and Bossj, M. (1992): Pap- and pil-related DNA sequences and other virulence determinants associated with *Escherichia Coli* isolated from septicemic chickens and turkeys. Infect. Immun. 60, 2648-2656.

El-Jakee, J.K.; Mahmoud, R.M.; Samy, A.A.; El-Shabrawy, M.A.; Effat, M.M. and Gad El-Said, W.A. (2012): Molecular characterization of *E. coli* isolated from chicken, cattle and buffaloes. Int. J. Microbiol. Res., 3(1): 64-74.

Ellakany, Hany F.; Hatem S. Abd. Elhamid; Ibrahim, MS.; Nagwa S, Mostafa and Ammed B, Gado (2019): Isolation, Serotyping, Pathogenicity and Antibiotic Sensitivity testing of *Escherichia Coli* from Broiler chickens in Egypt. JVS. 61(2):45-51.

Goren, E. (1990): Colibacillose bij pluimvee: etiologie, pathologie en therapie. pluimvee symposium. Upjohn-Nederland, Doorn, the Netherlands.

Goren. E. (1978): Observations on experimental infection of chicks with Escherichia Coli. Avian Pathol., 7: 213-224.

Gross, W.B. (1958): Symposium on chronic respiratory disease of poultry. II. The role of *Escherichia Coli* in the cause of chronic respiratory disease. Am. J. Vet. Res. 19, 448-452.

Jesus, E.B.; Miguel, B.; Azucena, M. and Jorge, B. (1997): Prevalence of bacterial resistance to quinolones and other Antimicrobials among Avian *Escherichia Coli* strains isolated from septicemia and health chickens in Spain. J. Clini. Micr. 2184-2185.

Jones, M.G.; Bokshi, K.N. and Mahanta, B. (2000): Isolation and identification, serotyping and antibiogram of *E. coli* mahaboobnagar district. Ind. Vet. J. 77: 4-6.

Khan, MS.; Akhtar, N.; Haque, ME.; Barua, A.; Chowdhury, T.; Mullick, R. and Mahmud, AM. (2014): Isolation and identification of non-plasmid multidrug resistant *E. coli* from poultry wastes in Chittagong, Bangladesh. J. Bacteriol parasitol 5: 182.

Kumar, N.; Kashyap, S.K.; Maharchandani, S. and Sharma, M.M. (2005): Characterization of *E. coli* strains associated with septicaemia in sheep. Ind. Vet. J. 82:6-8.

Melese, K. (2015): Isolation and characterization of bacteria associated with yolk sac infection (omphalitis) in chicks in Bishoftu poultry forms. Ethiopia. Msc Thesis. Addis Ababa University, college of veterinary medicine and Agriculture, Department of veterinary

microbiology, immunology and veterinary Public health.

Neter, E. (1959): Enteritis due to enteropathogenic *Escherichia Coli* present day status and unsolved problems. J. pediator. 55: 223-239.

Oteo, J.; Lizaro, E.; De Abajo, FJ.; Campos, I. and Spanish members of Earss (2005): Antimicrobial - resistant invasive *Escherichia Coli* Spain. Emerg. Infect. Dis. 11: 546-553.

Peighambari, S.M.; Vaillaneourt, J.P.; Wilson, R.A. and Gyles, C.L. (1995): Characteristics of *Escherichia Coli* isolates from avian cellulitis. Avian Dis. 39, 116- 124.

Petersen, A.; Andersen, J.S.; Kaewmak, T.; Somsiri, T. and Dalsgaarat, A. (2002): Impact of integrated fish farming on antimicrobial resistance in a pond environment. Appl. Environ. Microbial. 68. PP. 6036-6042.

Robert, H.; John, B.K.; Pawin, P.; Keiko, H. and Christina, Z. (2002): Prevalence of salmonella and *E. coli* and their resistance to antimicrobial agents, in farming communities in north Thailand. Medicine center, Michigan. St. Univ. East Lansing USA. (33): 120-126.

Schroeder, C.M.; Zhao, C.W. and Debroy, C. (2002): Antimicrobial resistance of *Escherichia Coli O157* isolated from humans, Cattle, Swine, and food. Appl. Environ. Microbial. 68:576-581.

Sojka, W.J. and Carnaghan, R.B.A. (1961): *Escherichia Coli* infection in poultry. Res. Vet. Sci. 2, 340-353.

Ulrich, L.; Antonia, R. and Timothy, S.C. (2008): Guideline for antimicrobial use in poultry, chapter8. Cityed by Guide Antimicrobial use in Animals. Edited by luca Guardabass, lars B. Jensen and Hilde Kruse.

Wen, YL.; Hui, YL.; Guang, CD.; Yong, Xz.; Shuai, YC.; Hai, YY. and yuan, LX. (2018): Emeregance and mechanism of carbapenem- resistant *Escherichia Coli* in Henan, China. Jour. Infect. Public. Health. 11: 347-351.

Whittam, T.S. and Wilson, S.A. (1988): Genetic relationships among pathogenic strains of avian Escherichia Coli. Infect. Immun. 56, 2458-2466.

White, D.G.; Wilson, R.A.; San Gabiiel, A.; Saco, M. and Whittam, T.S. (1990): Genetic relationships among of avian *Escherichia Coli* associated with swollen- head syndrome. Infect. Immin. 58, 3613-3620.

White, D.G.; Wilson, R.A.; Emery, D.A.; Nagaraja, K.V. and Whittam, T.S. (1993): Clonal diversity among strains of *Escherichia Coli* incriminated in turkey Colisepticemia. Vet. Microbiol. 34, 19-34.

WHO (2014): Antimicrobial resistance global report on surveillance: 2014 summary.