

Isolation of Pathogenic *Escherichia coli* O78:K80 Serotype From Broiler Chicks with Spontaneous Pathological Conditions in Basra Province

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Abstract

Escherichia coli (*E. coli*) is responsible for a variety of disease conditions which cause high economic losses in poultry due to high mortalities, decreased food conservation rate and condemnation of whole affected carcass or organs after slaughter. In the present study, 50 out of 70 broiler birds which have been collected from diseased chickens were exhibited lesions of fibrinous perihepatitis, fibrinous pericarditis and/or airsacculitis .Birds with these lesions were subjected for bacteriological examination.. The examination revealed isolation of 23 *E. coli* isolates with incidence of 46%. Concerning the virulence factors , the Congo red binding activity of these isolates reveled detection of 6 positive isolates with incidence of 26.08%. Serotyping showed that out of 6 Congo red positive strains, only 3 strains were serologically typed and were belonged to the serotype O78:K80. Detection of pathogenic *E. coli* serotypes was confirmed by PCR technique with specific primers for *fimA* and *fimH* genes. All these 3 isolates of the serotype O78:K80 were reacted with these 2 genes. Antibiogram susceptibility pattern displayed sensitivity of these isolates to Chloramphenicol and Gentamicin and their resistance to Amoxicillin ,Erythromycin and Nalidixic acid.

Key words: *Escherichia coli*, serotyping, virulence, PCR.

عزل النمط المصلي O78:K80 للايشريشيات القولونية الممرضة من أفراخ فروج اللحم المصابة ببعض التغيرات المرضية في محافظة البصرة.

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الخلاصة:

تعتبر الايشريشيات القولونية مسؤولة عن إحداث حالات مرضية مختلفة تسبب خسائر اقتصادية ناتجة عن الهلاكات العالية وانخفاض معدل التحويل الغذائي والخسائر في إتلاف الجثث والأعضاء المصابة بعد الذبح. تم في الدراسة الحالية فحص 70 عينة من أفراخ فروج اللحم الهالكة أو التي كانت على وشك الموت وقد تبين ان 50 منها كانت تعاني من التهاب الكبد الفبريني أو التهاب التامور والأكياس الهوائية الفبريني. و بعد الفحص الجرثومي لهذه العينات تم عزل ثلاثة وعشرين عذلة من جراثيم الايشريشيات القولونية أي بنسبة % 46 وبعد إجراء فحص الضراوة باستعمال الكونكو الأحمر تم الحصول على ست عزلات ممرضة وتأكد ذلك من خلال الفحص المصلي باستعمال مصل مضاد خاص بالنمط O78:K8 إن ثلاثة فقط تعود إلى هذا النمط المصلي وقد

اجري فحص الحساسية الدوائية لهذه العزلات الستة وتبين حساسيتها لكل من الكلورامفينكول و الجنتاماسين ومقاومة للاموكسلين والارثرومايسين و ناليدكسك اسد. كما تم تأكيد عزل النمط المصلي O78:K80 باستعمال تقنية PCR حيث اتضح تفاعل العزلات الثلاثة مع نوعين من الجينات الممرضة وهما *fimH* و *fimA* جين.

Introduction:

Most strains of *Escherichia coli* (*E. coli*) are harmless commensal organisms, but any strain of *E. coli* can cause disease if penetrates the gut mucosa and enters the blood stream. When *E. coli* reach blood, it releases endotoxins that cause fever and even death (Ezz El Deen *et al*, 2010).

Avian pathogenic *E. coli* (APEC) is associated mainly with extraintestinal diseases in chickens, turkeys, and other avian species and causes severe economic losses to the poultry industry. The most common form of these diseases is avian colibacillosis, which starts as a respiratory infection (airsacculitis) and is frequently followed by generalized infections such as perihepatitis, pericarditis, and septicemia. APEC belongs to a limited number of serogroups, of which O1, O2, O78 are the most common (La Rajione *et al*, 2002).

Avian colibacillosis is an economically important infectious disease of domestic poultry, and the most commonly implicated serotypes are O1:K1, O2:K1, and O78:K80. The most severe clinical manifestation of *E. coli* infections in poultry is colisepticemia which often begins as an upper respiratory infection following a predisposing primary bacterial or viral infection and leads to infiltration of the blood and internal organs with development of pericarditis, perihepatitis, airsacculitis and salpingitis (Amara *et al*, 1996).

Strains of *E. coli* serotype O78:K80 are associated with a large variety of diseases,

including invasive infections. O78:K80 strains are the causative agents of diseases in farm animals, causing

sepsis in sheep and poultry. Around the world the disease is

commonly associated with serotype O1, O2, and O78, with the latter two constituting

about 80% of the cases (Adiri *et al*, 2003). The present study was aimed to isolate *E. coli* strain associated with chicken infection, precisely :pericarditis, perihepatitis, and airsacculitis to study the characteristics of such strain by serotyping with monovalent specific O78:K80 antisera and detection of virulence factors by Congo red activity and genes by PCR assay, and finally determination of antimicrobial susceptibility pattern of the recovered *E. coli* strains.

The article is part of the first author thesis.

Materials And Methods

Dead and morbid broiler chickens of different ages and localities of Basra farms were subjected for post-mortem examination. After necropsy, 50 out of 70 examined birds were exhibited different degrees of fibrinous perihepatitis fibrinous pericarditis,, and/or airsacculitis. Sterile cotton swabs have been taken from cheesy material on the liver surface and inoculated in brain heart broth and subjected for bacteriological examination in an attempt to isolate *E. coli* (Ezz El Deen *et al*, 2010).

Isolation and identification of *E. coli* were performed according to Quinn *et al*.(2002). Briefly, loopfull from the broth was streaked on MacConkey

agar (Titan media/India) and incubated at 37 c° for 24 hours. The suspected lactose fermenting red colonies were picked up and examined for their biochemical reactions. API20 E system (BIOMERIEUX/France) was used for confirmation .

Detection of virulence factor by Congo red binding activity has been performed according to Berkhof and Vinal (1986). All *E. coli* isolates were tested for their growth on Tryptic Soya Agar media (Himedia/India) supplemented with 0.03% Congo red. The reaction was noticed after 24 hours of incubation at 37c° and then left at room temperature for additional 2 days. The Congo red positive (CR+) *E. coli* was identified as red colonies while the Congo red negative (CR⁻) *E. coli* appear white or colorless.

Serological examination was performed by slide agglutination test using specific O78:K80 antisera (PLASMATEC/URS) according to the manufactures instructions.

Antimicrobial susceptibility testing pattern of the serological positive *E. coli* isolates was determined by applying disc diffusion technique according to Salehy and Bonab (2006).

Detection of virulent genes of the pathogenic isolated *E. coli* (CR+) was performed by Polymerase Chain Reaction (PCR) with specific primers for *fimA* and *fimH* genes according to Vandemaele *et al.*, (2003). Briefly, the DNA of the 6 Congo red positive isolates were extracted and amplified. The amplified reaction was performed in PCR tubes, the reaction mixture consist of extracted DNA template from bacterial culture and specific primer reagents. The first primer sequence is:

fimAF (forward primer): 5' ACTGTGCAGTGTGGCAG 3'.

fimAR (reverse primer): 5' GTTATTTTTATCGCACAAGG 3' . and the amplified product size is 549 to 555 bp, while the second was:

fimHF: 5' ATGAAACGAGTTATTACCCTGTT TG3'

fimHR: 5' TTATTGATAAACAAAAG TCACGCC3'.

and the amplified product size is 903 bp. The first sequence was designed for amplification of *fimA* gene while the second was used for *fimH* gene. The electrophoresis was done and gel was examined under short wave UV transilluminator.

Results And Discussion

In bird *E. coli* is a part of the common microflora of their intestine and most isolates are nonpathogenic. About 10 to 15 % of the intestine coliforms are pathogenic serotypes (Barnes and Gross, 1997). *E. coli* is a ubiquitous organism in worm-blooded animals and chickens and regarded as major pathogen of worldwide importance in commercially produce poultry (Geornaras *et al*, 2001).

Avian colibacillosis is an infectious disease of bird caused by *E. coli*, which consider as one of the principle causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease condition, either as primary or as secondary pathogen. Although *E. coli* is normal inhabitant of intestinal tract of birds, it is capable of producing disease under influence of predisposing factors like overcrowding, inadequate ventilation, thirst and extreme temperature (Kabir, 2010).

In the present study, post-mortem examination displayed that 50 out of 70 examined birds, were exhibited pathological changes ranging from mild to severe fibrinous

pericarditis, fibrinous perihepatitis and/or airsacculitis. Bacteriological examination of these fibrinous material revealed isolation of 23 *E. coli* isolates with incidence of 46%. This result was in disagreement with that of Barbour *et al*, (1985) and Ezz El Deen *et al*, (2010), who isolated *E. coli* from chickens with incidence of 67.7% and 75% respectively. These variations may be due to the site of sample collections or may be due to geographical situations. In India Mohany *et al*, (1979) recorded higher incidence of 88.8%. On the other hand the result of the present study was in agreement with that of Ramaswamy *et al*, (1982) who identified these bacteria from diseased chickens with incidence of 47.3%, and in the same line of that of Sripoernomo *et al*, (1992) who stated that the incidence was 34.3%. Virulence of strains, route of infection, age of infected bird, dose of bacteria, immune status and predisposing factors may have a role in these variations of incidence. Other microbial agents may be implicated in producing pathological changes in *E. coli* negative cases.

Concerning the virulence factors of *E. coli*, the Congo red binding activity of the isolates, the present study revealed detection of 6 positive isolates with incidence of 26.08%. This result was in disagreement with that of Ezz El Deen *et al*, (2010) who stated that 100% of *E. coli* isolates from diseased chickens was (CR+). Berkhoff and Vinal, (1986) speculated that Congo red dye bounded to bacterial surface component might be required for this ability or were linked to undescribed virulence factor of *E. coli*. They also found the stability of Congo red binding activity was greater in some isolates than in the others and (CR+)

isolate lost the binding to Congo red dye if cultured on the blood agar. Harry and Yoder (1989) were used Congo red medium to determine the pathogenicity of *E. coli* isolates obtained from chicken and found the same result. In this investigation serotyping of *E. coli* isolates was determined by specific monovalent antisera for O78:K80 which showed that 3 out of 6 pathogenic *E. coli* isolates were O78:K80 positive with incidence of 50%, whereas the other 3 (50%) isolates were undetermined. This result was greatly differed from that of Ezz El Deen *et al*, (2010) who recorded O78:K80 serotype in an incidence of 3.45% from diseased chickens. The percentage of untypable *E. coli* strains of the present study was also in disagreement with that of the same researchers who recorded 26.67%. Kim and Namgoon (1987); Allan *et al* (1993) stated that the large percentage of untypable strains was common characteristics of all groups of *E. coli* recovered from avian colibacillosis regardless of geographic location, and the very low frequency of isolation of some serotypes known worldwide to be pathogenic for avian species is unknown and need further investigations by studying a higher number of isolates to be able to make definitive conclusion.

One of the aims of the present study was to detect the antimicrobial susceptibility patterns of *E. coli* serogroups by disc diffusion tests to determine the most preferable antimicrobial agents and to detect the antimicrobial resistance of isolated strains. The antimicrobial sensitivity testing for the examined isolates recovered from diseased chickens were shown in Table 1 below.

Table 1, The percentage of sensitivity and resistance of 6 pathogenic *E. coli*
Table 1 displayed that pathogenic *E. coli* isolates showed higher incidence of

| Antimicrobial agents | Disc content mg | Diffusion zone breakpoint (mm) | Percentage of Sensitive | Percentage of Intermediate | Percentage of Resistant |
|-----------------------------|-----------------|--------------------------------|-------------------------|----------------------------|-------------------------|
| Ampicillin | 10 mcg | ≤ 13 | 17 | 0 | 83 |
| Amoxicillin\clavulanic acid | 30mcg | ≤14 | 0 | 0 | 100 |
| Chloramphenicol | 30mcg | ≤ 12 | 67 | 0 | 33 |
| Erythromycin | 15mcg | ≤ 13 | 0 | 0 | 100 |
| Gentamicin | 10mcg | ≤ 14 | 50 | 17 | 33 |
| Nalidixic acid | 10mcg | ≤ 13 | 0 | 0 | 100 |
| Penicillin | 10mcg | ≤13 | 0 | 50 | 50 |

sensitivity to Chloramphenicol and Gentamicin and resistance to Amoxicillin\clavulanic acid, Erythromycin, and Nalidixic acid. This result was in agreement with that of Guerra *et al.*, (2003); Saenz *et al.*, (2003) who found that *E. coli* isolates were sensitive to Chloramphenicol and Gentamicin and resistance to Amoxicillin\clavulanic acid ,Erythromycin and Nalidixic acid. *E. coli* isolates were resistant to these drugs because of their regular usage in poultry industry for control of avian colibacillosis. The result of the present study was in disagreement with that of Salehi and Bonab,(2006) who found that *E coli* isolates were sensitive to Gentamicin and resistance to Chloramphenicol,

Amoxicillin\clavulanic acid, Erythromycin and Nalidixic acid.

Polymerase Chain Reaction (PCR) has emerged as a rapid and sensitive diagnostic tool for detection small quantities of target DNA(Chapman *et al.*, 1997). PCR providing an alternative tool for rapid, sensitive and accurate diagnosis, as well as for epidemiological studies, and for molecular characterization of serotypes (Ewers *et al* 2005).

DNA extraction from pathogenic *E. coli* isolates, in the present study, was used in PCR assay to detect the presence of O78:K80 serotype using *fimA* and *fimH* genes sequences to allow discrimination O78:K80 serotype from other serotypes in field samples of commercial poultry. The

detection of pathogenic *E. coli* by Congo red and specific antisera was confirmed by PCR using 2 fimbriae genes revealed 2 bands with

amplification size of 549-555 and 903 bp to both two genes, as shown in the photograph below:

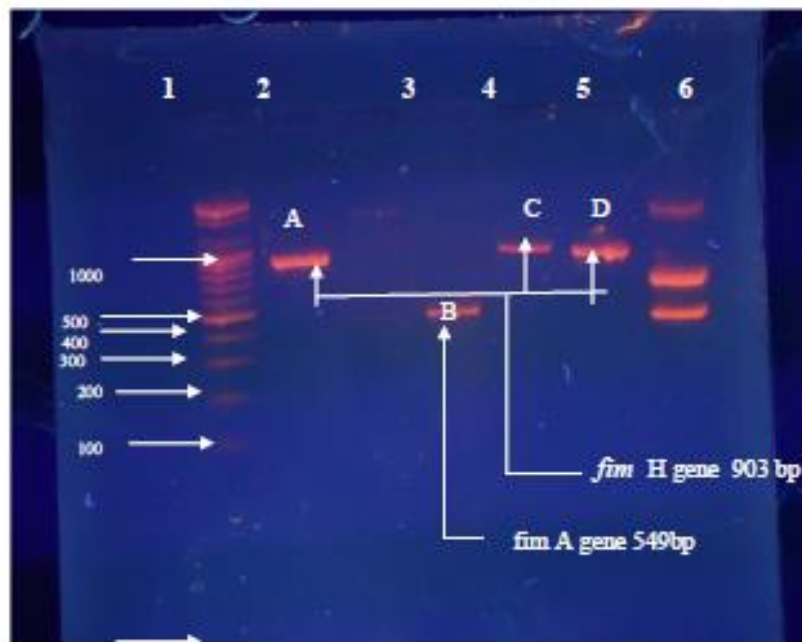


Figure (1): PCR amplification of *fimA* gene (549-555 bp) and *fimH* gene (903 bp) Lanes 2, 4, 5(A, C, and D isolates) were positive to *fimH* gene, Lane 3(B isolates) was positive to *fimA* gene. Lanes 1, 6 were ladders.

The six pathogenic *E. coli* isolates named A, B, C, D, E, and F in the present study were tested by PCR. Fig. (1) demonstrated the results of 4 isolates (A, B, C, and D).

Table 2: APEC for two genes PCR amplification

| Pathogenic <i>E. coli</i> isolates | <i>fim H</i> (903) | <i>fim A</i> (549-555) |
|------------------------------------|--------------------|------------------------|
| A | + | + |
| B | + | + |
| C | + | - |
| D | + | + |
| E | + | + |
| F | + | + |

Table 2 above showed the classification of the isolates as APEC was based on detection of some virulent factors., Vandemaele *et al.*,(2003) reported that the multiplex PCR technique highlighted two genes, *fimH* and *fimA* genes, as APEC specific virulent factors encode a fimbrial adhesin . The result of present study was in concordance with the finding of this author who stated that *E .coli* O78:K80 serotype isolated from diseased chickens was reacted with both *fimA* with amplification size 549-555 and with *fimH* with amplification size 903 bp. La Ragione *et al* (2002) studied the role of fimbriae in adherence of avian strains of *E. coli* O78:K80 to tissue culture cells ,tracheal and gut explants and found that lacking of fimbriae reduced adherence at 90 % in comparison with of those which have fimbriae.

Pourbakhsh *et al.* ,(1997) demonstrated the involvement of type 1 fimbriae in the colonization of the upper respiratory tract in experimentally inoculated chickens and suggested that P fimbriae may be involved in the colonization of internal organs and in the development of septicaemia. Vandemaele *et al.* ,(2003)were analyzed the sequence of *fimH* and *fimA* genes in 24 isolates of APEC and demonstrated that *fimH* is a conserved adhesin, while *fimA* presents a variable sequence.

The ability of bacteria to adhere to host epithelial cells is considered to be a prerequisite for the establishment of infectious diseases, mainly through expression of fimbriae, avian pathogenic *E. coli* (APEC) generally possess type 1 and P fimbriae, type 1 fimbriae are characterized by the ability to agglutinate chicken

erythrocytes.They consist of a major protein, FimA, FimF, FimG and the adhesin

FimH, encoded by the *fim* gene cluster . This type of fimbria is common among

Enterobacteriaceae and several variants have been associated with APEC, it has

been suggested that they may be involved in the initial stages of

colonization of upper respiratory tract(Knobl *et al.*,2006)

Therefore continuous researches especially that use DNA related techniques have proved to be useful in discriminating the pathogenic isolate of *E. coli*, which commonly belong to certain serogroups, particularly the group O78:K80.

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