

DETECTION OF VIRULENCE AND ADHERENCE GENE IN *Escherichia Coli* O157:H7 ISOLATED FROM ANIMAL PRODUCTS

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ABSTRACT

Twenty nine *E. coli* isolates were previously isolated from beef from slaughter house, and raw milk of different origin were collected from different parts in Basrah. Five *E. coli* isolates were identified as *E.coli* O157:H7 using O157 and H7 antisera. *E. coli* O157:H7 isolates were tested for antimicrobial susceptibility test. The results showed that all tested bacteria were sensitive to gentamycin and Amikacin and resistant to amoxicillin and cefoxitim. The DNA of these isolates were amplified to detect verotoxins gene (*vt1*, 91bp) and pyelonephritis-associated pili gene (*pap*,91bp). All the isolates were observed having (*vt1*) All the isolates were observed having (*vt1*) and *pap* genes.

INTRODUCTION

Escherichia coli, a short-rod shaped, Gram-negative facultative anaerobic bacterium commonly found in intestinal tracts of human and warm-blooded animals. Most of the *E.coli* present in the gut flora is harmless symbiont but sometimes they have the ability to cause wide range of human and animal diseases like mild diarrhea, cholera-like diarrhea and invasive dysentery (1).

Based on differences in antigenic structure on the bacterial surface *E. coli* can be characterized by serotyping, these antigens are broken into three major groups (2). These three groups are O, which is somatic antigen, H, the flagella antigen group, and K, which is the capsule antigen group (3). Serotyping is an important tool which can be used in combination with other methods to distinguish pathogenic *E. coli* strains as specific pathogenicity attributes are often linked to certain serotypes. (4; 5).

However, some subsets of *E. coli* have acquired specific virulence characteristics that let them capable of causing a variety of diseases in healthy humans and animals. Most

acquired virulence factors that distinguish pathogenic *E. coli* from commensals are encoded by mobile genetic elements such as plasmids, bacteriophages and transposones (6; 7). The pathogenic *E. coli* are divided into different pathotypes according to the virulence factors they possess. *E. coli* O157:H7 serotypes are identified as enterohaemorrhagic *E. coli* EHEC and categorized as verotoxin-producing *E. coli*. verotoxin is also known as shiga-like toxin, human and bovine *E. coli* O157:H7 elaborates two potent phage encoded cytotoxins, known as Shiga-toxins *Stx1* and *Stx2* or verotoxins *VT1* and *VT2* (8; 9).

Pyelonephritis-associated fimbriae (P fimbriae) are known to play an essential role in the pathogenesis of Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *E. coli* (UPEC) infections by means of their PapG adhesion. These findings suggest that P fimbriae are involved in the virulence of APEC O1 (10). P fimbria a mannose-resistant adhesion of UPEC. Pap gene cluster encodes the proteins required for P-fimbrial biogenesis, including papG, which encodes the tip adhesion (11). Pap gene used in this study coding for pathogenicity islands (PAI I, PAI II) and *sfp* gene cluster. The majority of *Escherichia coli* strains isolated from urinary tract infections have the potential to express multiple fimbriae. Two of the most common fimbrial adhesins are type 1 fimbriae and pyelonephritis-associated pili (12).

This study was conducted to investigate the presence of virulence and adherence genes (*vt1* and *pap*) in *Escherichia coli* O157:H7 isolated from beef and raw milk.

MATERIALS AND METHODS

Bacterial strains

Twenty nine *E. coli* were previously isolated from beef from slaughter house and raw milk of different animals were collected from different parts in Basrah. All *E. coli* isolates were screened on sorbitol MacConkey (supplemented with cefixime 0.05 mg/l) agar plates, cellobiose fermentation and KCN broth turbidity test. *E. coli* isolates also were serotyped by slide agglutination technique using O157 and H7 antisera (Murex Wellcolex, UK).

Antimicrobial susceptibility testing: Disk diffusion method on Mueller-Hinton agar was used according to (13). Antimicrobial disks used were: amoxicillin, amikacin, gentamicin, cefoxitin and ciprofloxacin.

Detection of *Vt1* and *Pap* genes: Molecular Detection of verotoxine gene *Vt1* and *Pap* genes by using PCR assay was done by using commercially available DNA extraction and purification kit (Geneaid, USA). The purified DNA was detected by electrophoresis in 2% agarose gel supplemented with ethidium bromide. Methylene blue stain added to the DNA sample and the DNA was visualized under U.V. light.

Primer used for *E. coli*

Vt1F CGCATAGTGGAACCTCACTGACGC 91bp (14)

Vt1R CATCCCCGTACGACTGATCCC

PapF CCGGCGTTCAGGCTGTAGCTG 97bp (14)

PapR GCTACAGTGGCAGTATGAGTAATGACCGTTA

The verotoxin genes were studied according to protocol of (14). This was done by using customize primers. The PCR reaction mixture contains 5 µl of green master mix (contains bacterially derived *Taq* DNA polymerase, dNTPs, MgCl₂ and reaction buffer at optimal concentration for efficient amplification of DNA templates by PCR), 5 µl of purified bacterial DNA, 1 µl of each forward and reverse primers, then the volume completed to 20 µl by deionized water. All tubes were centrifuged in microcentrifuge for 10 seconds. The PCR tubes were transferred to the thermacycler to start the amplification reaction according to specific program for each gene. The agarose gel was prepared according to the method of (15). Two concentrations of agarose gel were prepared (1% and 2%) as we needed. The concentration of 1% agarose was used in electrophoresis after DNA purification process, while 2% agarose was used after PCR detection.

Ten microliters from amplified sample was directly loaded in a 2% agarose gel containing 0.5 µl /25ml ethidium bromide with the addition of loading buffer and DNA size. Marker as standard in electrophoresis and the gel was run at 75 V. at 1 hr, then the products were visualized by UV transilluminator.

RESULTS

Table (1) show that all of *E. coli* isolates were identified as non sorbitol fermenter, negative to cellobiose fermentation and KCN broth turbidity. It showed also that *E. coli*

O157:H7 was found at the percentage of 16.6% from beef, 33.3% of milk and 23.8% of total isolats among nonsorbitol fermenting *E. coli* after serological test.

Table 1. Biochemical and serological tests of *E. coli* isolates

Samples origin	No. of <i>E. coli</i> isolates	Sorbitol fermentation	Cellobiose fermentation	KCN broth turbidity	Serotype <i>E. coli</i> O157:H7
Beef	12	-	-	-	2 (16.6%)
Milk	9	-	-	-	3 (33.3%)
Total	21				5 (23.8%)

Antimicrobial Susceptibility Test

Five *E. coli* O157:H7 isolates were tested for antimicrobial susceptibility test (fig.1). The results showed that all tested bacteria were sensitive to gentamycin and Amikacin , most of them were sensitive to ciprofloxacin, and all were resistant to amoxicillin and ceftiofur.

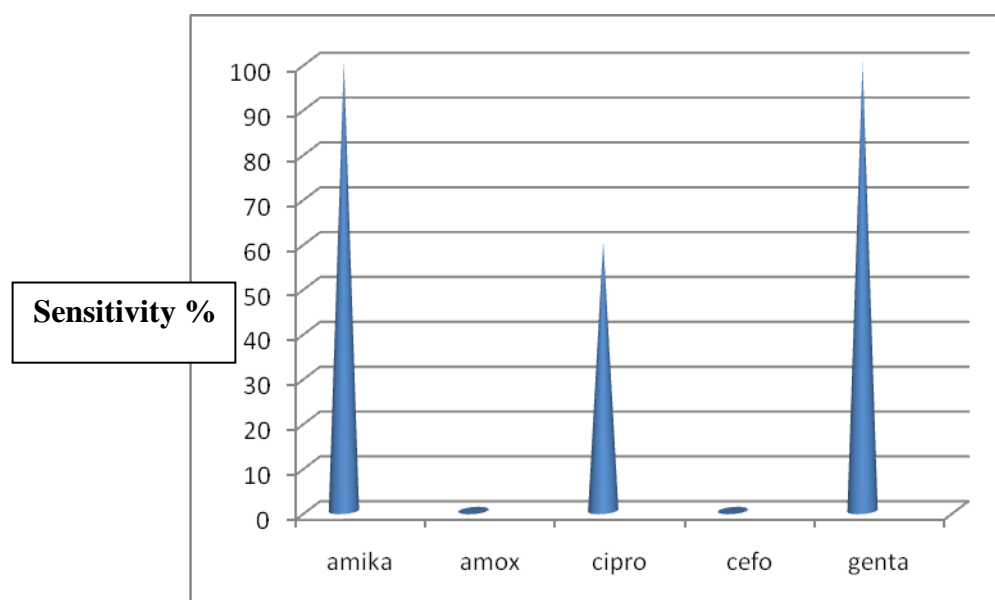


Figure 1. Antimicrobial sensitivity tests for *E. coli* O157:H7 isolates against different antibiotics

Detecting of virulence and adhesion genes by PCR

The DNA of all isolates was extracted and purified by using genomic DNA purification kit. All the isolates which were positive for *E.coli* O157:H7 latex agglutination test were amplified to detect of *vt1* and *pap* genes. *vt1* gene (about 91bp) was observed in 100 % (5/5) of the tested isolates (Fig. 2). At the similar manner all five isolate of *E.coli* O157:H7 were observed having *Pap* gene (about 97bp) in a 100 % of isolates (figure 3).

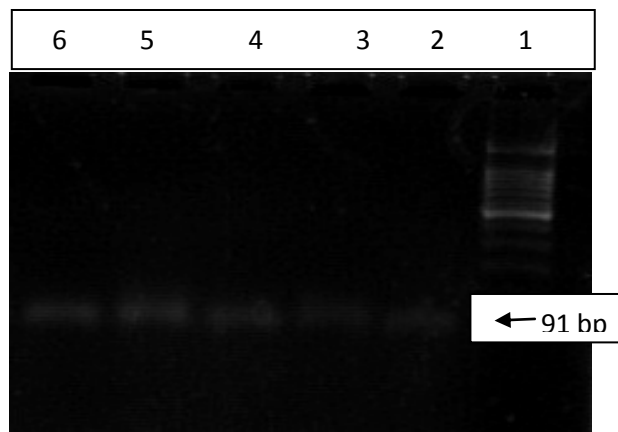


Figure (2): PCR amplification of *VT1* gene, 91bp (Lan 2-6), Lane 1= 1000 bp ladder.

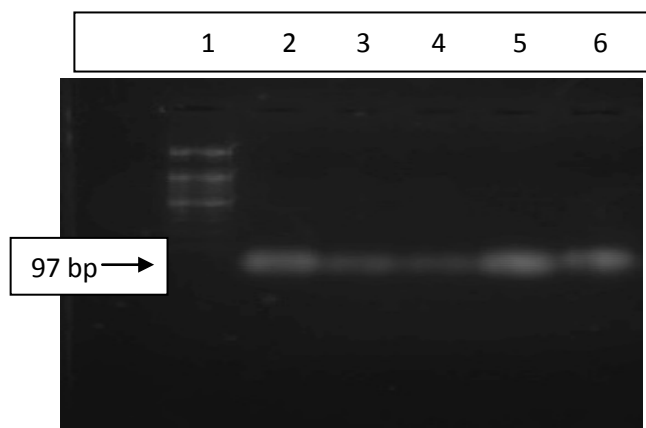


Figure (2): PCR amplification of *Pap* gene, 97bp (Lan 2-6), Lan1 = 1000 bp ladder.

DISCUSSION

Previous studies showed that shiga toxin producing *E. coli* O157:H7 was isolated from 3.7% beef and 1.5% of prok samples in United States and Canada (16). Although most sporadic cases and out breaks have been recorded from developed countries, human infections associated with STEC strains have also been described in latin American countries (17). It has also been reported from Kenya, Turkey and Iraq (18). Many studies determined the prevalence of *E. coli*O157:H7 on cattle which were from 0.0% to 27% (up to 68% in heifers), (19).

The present study showed that 16.6% of raw beef samples were contaminated with *E. coli* O157:H7 .Our result suggested that cattle could be a reservoir of *E. coli* O157:H7 in Iraq, like many countries (20).The ability of this study to detect serotype O157:H7 in lower rates among non –sorbitol fermenting *E. coli* isolates in beef and milk confirms the results obtained by other author, who reported that this serotype is uncommon and its isolation rates are much lower than those of non O157:H7 serotypes (21). On contrary, Wells *et al.* (22) determined the prevalence of *E. coli* O157:H7 and found that this organism was isolated from 5 of 210 calves (2.3%) .Surveys of United states dairy and beef have found *E. coli*O157:H7 in 0 to 2.8% of animals. All isolates of bacteria showed sensitive (100%) to amikacin, and gentamycine and totally resistant to amoxicillin and cefoxitim. The continued overwhelming sensitivity of *E. coli* O157 in this study to some antibiotics tested is astonishing considering the rapid increases in resistance found in other zoonotic bacteria such as *Salmonella* spp., and *Campylobacter* spp. (23).

The occurrence of PCR positive for *vt₁* of *E.coli* O157:H7 isolates from raw milk samples (100%) was much higher from that registered in Spain (0.4%) by Quinto and Cepeda, (24), in Ontario (0.87%) by Steel *et al.* (25), in Egypt (2.6 % and 1.10%) by Hassan and Elmalt (26) and El-Safey (27) respectivley and in Germany (3.9%) by Klie *et al.*, (28). The high rate recorded in the present study can be attributed to the use of primers designed to target genes *vt₁* which encoded for Vt1 toxins (29). The detection of VTEC strains in the foods of animal origin including raw milk and its products have been implicated a health risk to consumers or as important vehicles for VTEC infections in humans (30).

All the isolates which were positive for *E.coli* O157:H7 latex agglutination test were amplified to detect of *vt₁* and *pap* genes. *vt₁* gene (about 91bp) was observed in percentage100 % (5/5) of the tested isolates. At the similar manner all five isolate of *E.coli*

O157:H7 were observed having *Pap* gene (about 97bp) in a percentage 100 %. *Pap* gene is one of the the most common fimbrial adhesins called type1 fimbriae and pyelonephritis-associated pili (*Pap*), (12).

التحري عن جينات الضراوه والالتصاق في جرثومة الاشيريشيا القولونية O157:H7 المعزوله من المنتجات الحيوانيه

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الخلاصه

تم دراسة 29 عزله من الاشيريشيا القولونية المعزوله سابقا من لحوم الحيوانات المذبوحه في المجزره ومن الحليب الخام من مصادر مختلفه ومن مناطق مختلفه من البصره. خمسه منها تم تشخيصها كونها الاشيريشيا القولونية O157:H7 باستخدام المضادين المصلين O157 و H7. تم اختبار هذه العزلات لحساسيتها للمضادات الحيويه وقد اشارت النتائج بان كل الجراثيم المختبره حساسه للجنتاميسين والاميكاسين ومقاومه للاموكسيسيلين والسيفوكسيتيم. تم تضخيم الدنا لهذه العزلات لاختبار وجود جين الضراوه والسميه (*vt1*, 91bp) والجين (*pap*, 91bp) الخاص بالاهلاب. وقد لوحظ تواجد الجينين في كل العزلات المختبره.

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