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Application of chromogen culture media and PCR for detection ofE.coli O157:H7and flicH7, rfbO157Genes respectively inHumans and Dogs isolates

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Abstract: *EnterohemorrhagicE.coli (EHEC) O157:H7 is ahuman pathogen responsible for outbreak of bloody diarrhea and hemolytic uremic syndrome worldwide polymerase chain reaction was used to investigate the genotypic properties and genetic relationship between E.coli O157:H7 obtained from diarreheal humans and dogs cases in Iraq. 88 (88%) E.coli isolates were isolated from 100 stool sample of humans and 86 (86%) isolates from 100 fecal samples of dogs by conventional methods (culture, biochemical tests and chrom agar). A total of 174 E.coli isolate from human and dog were subjected to PCR to detect the flicH7 gene and rfbO157 gene by amplifying a 625-bp region for flicH7 and a259 bp region for rfbO157 gene using the same primers. Results showed that, 4(4.5 %) and 4 (4.7 %) isolates from humans and dogs respectively give positive product for flicH7 gene and rfbO157 gene respectively. These results suggested that, there is a genetic relationship*

Keyword: *E. coli O157:H7, flicH7gene, rfbO157gene, zoonosis, K9 dogs*

I. Introduction

Dogs are the most successful canids, acclimatized to human habitation worldwide. They have contributed to physical, social and emotional well-being of their owners, **{10,19}**.Domestic dogs have long been recognized to be a potential source of zoonoses for people**{21,8}**In particular, zoonotic bacteria and parasites harbored in the canine intestine have been shown to pose a significant risk to human health**{21,8}.**

People are exposed to these pathogens through direct or indirect contact with infected dogs or their feces, and they may become infected after inadvertent ingestion of a zoonotic agent**{16,8}**.Although there are more than 300 recognized canine Zoonosis **{7,13}**,alimited number of these are caused by bacterial agents and have dogs as the main host species,however, these have a significant public health impact as some of them, though to be mild are widespread while others can be severe or even fatal.**{6}**.suchas: *Escherichia coli(O157:H7).*

E. coli O157:H7 and other Enterohemorrhagic serotypes have emerged as major food-borne, zoonotic pathogens in humans, responsible for the hemorrhagic colitis-hemolytic uremic syndrome **{17}**.

E.coli O157:H7 was the main of serotype EHEC gram-negative, rod (bacillus) belong the family of enterobacteriaceae, and this pathogen was considered a predominant serotype of Shiga toxin producing E. coli (STEC) **{3,25}**.

lacking studies on E.coli O157:H7 recovered from K9 dog especially in Iraq, also for the role of K9 dog in detecting and decreasing terrorist activities,and for many people who are working in management and breeding of K9 dogs, the study aimed to confirmidentification of E.coli O157:H7 in K9 dogs and humans, andclarify genetic similarity of most important strain which were isolated from the humans and dogs by application of PCR.

II. Methodology

2.1. Collection of samples

One hundred dogs fecal sample, 46 Belgium dogs (5dogs from Babel and 41 from Baghdad), 3Black-Walf dogs from Baghdad, 50German shepherd-dogs (37 from Baghdad, 12from salahaldeen, and 1 from babel),1Rott wailer dog from Baghdad. Veterinary clinic in tuz and ministry of entry were also sites included in the study.

Alsoone hundredhuman's fecal sample which were collected from outpatient of Tuz hospital, suffering from diarrhea.both humans and dogs fecal samples were collected and transmitted immediately to the laboratory, or by using ice-cooled box to the laboratory for bacterial culture.

2.2. Phenotypic identification by culturing forlaboratory diagnosis ofE. coli

Samples were cultured initially onMacConkeyagar and incubated at 37°C for 24hours, then subcultured on Eosin Methylene blue agar and incubated aerobically at 37 °C for 24hours**{18}**.

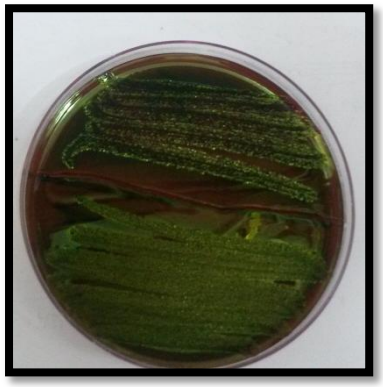


Fig1.Escherichia coli onEMB agarConventional Biochemical Tests:

Biochemical tests were performed according to **{5**}.

Culturing on CHROM agar

A typical colonies onSorbitol MacConkey agar(non-Sorbitol fermented *E. coli*) were streaked on chromo agarplatesand incubated at 37˚C for 24 hours .The colonies of *E.coli*O157 were appeared as mauve colonies while non-pathogenic*E. coli* appeared as blue colonies.

Figure (2): *E.coli*O157:H7 showedMauve color colonies in chromagarO157 at 37°C for 24hrs.

Figure (3): *E.coli*showed a blue color On chromagarO157 at 37C^{o}for 24hrs

2.3Genotypic identification Polymerase Chain Reaction (PCR) assay:

1: DNA extraction

Genomic DNA of *Escherichia* coli isolatewas extracted by using (Genomic DNA Mini Kit, Geneaid. USA)

2:Primers selection

All the primers in this study were obtained from Bioneer,Korea.These primers were used to detect E.coli O157:H7 at genus level. These primers were prepared according to the information's of the company. FlicH7 gene was designed by **{22}**, while rfbO157 gene was designed by **{11}**.the sequence of the primers which were used in this study is showed in table(1)

Table(1) sequence of primers for FlicH7 gene and rfbO157 gene.

Primer	*Sequence(Sequence (5' – 3')*		*Product Size*
flicH7	*F*	*GCG CTG TCG AGT TCT ATC GAG*	*625 bp*
	R	*CAA CGG TGA CTT TAT CGC CAT TCC*	
rfbO157	*F*	*CGG ACA TCC ATG TGA TAT GG*	*259bp*
	R	*TTG CCT ATG TAC AGC TAA TCC*	*259bp*

3. Amplifications:

The PCR amplification mixture (20µl) which was used for detection the genes includes master mix, which provided by Bioneer (Korea) include 5µl of template DNA , 1.5µl of each forwarded and reversed primers and 12µl of nuclease free water to complete the amplification mixture to 20µl.the PCR conditions started with thermo-cycler program, showed in table(2)

Table (2) PCR thermo-cycler condition for E.coligenes**:**

Thermocycling	Primers *flicH7* *rfbO157*
Initial denaturation	**94°C/5min**
PCR Amplification cycle*	
Denaturation	**94 °C /1 min.**
Annealing	**52°C/ 45 sec.**
Extention	**72 °C/ 90 sec.**
Final extention	**72ºC/10 min.**

*Repeat the step (35 cycles)

4:PCR Product Analysis (Agarose Gel Electrophoresis):

It is a very important step to complete PCR assay ,which was used to analyse the PCR product by 1.5%agarose gel electrophoresis supplied with 3µL of ethidium bromide and using 8µL of 100bp ladder, Finally PCR products (bands) were visualized using a UV trans illuminator and photographed by using digital camera.

III. Results and discussion:

1. PhenotypingE.Coli O157:H7

The bacteriological culturing revealed a green metallic sheen colonies on eosin methylene blue agar,also these colonies showed mauve color on ChromagarO157 agar, and the bacterial isolates expressed gram-negative stain, this features may be indicated that the bacterial colonies belonged to *E.coli*O157:H7,this result was similar to bacterial colonies of *E.coli*O157:H7 that recorded by **{24,1}.**

Biochemical tests results were showed as in Table(3),that the isolates were positive for indole test , MR test, oxidase test and catalase test, while they were negative for VP, Simmon's citrate, and urease tests and acidic yellow in both top and bottom of TSI.

Table (3) shows: - biochemical tests results

Test	Result
indole	**Positive**
MR	**Positive**
Catalase	**Positive**
Simmon Citrate	**Negative**
VP	**Negative**
Urease	**Negative**
TSI	**Acidic/Acidic**

The bacterial isolation and identification showed that 88(88%), out of 100 human stool samples were *E. coli* positive and 86(86%) out of 100 dog fecal samples *E. coli* positive. After initial isolation and identification of *E. coli* on general, selective and biochemical test.

The isolates of *E. coli* O157:H7 were appeared with mauve color on Chrom agar and other *E.coli* appeared in blue color,Figures (2, 3).Chrom agar aids in diagnosis of *E.coli* 0157:H7.*E. coli* O157:H7 utilizes one of chromo genic substrates which produce mauve colored colonies.

While non- *E. coli* O157:H7 organism may utilize chromo genic substrates resulting in blue to blue green colored colonies,this result agreed withthe resultsmentioned by ,**{15**,**25** and**26}**.These isolates were identified and confirmed by multiplex PCR assay, this test showed that 4(4.5%) out of 88 *E. coli* isolates of human stool were *E. coli* O157:H7 positive, while 4(4.7%) out of 86 *E. coli* isolates were *E. coli* O157:H7 positive in dog fecal samples isolates. **(14)**reported the rate of infection which was higher (3.84 %) among children <2 years of age than other age groups. also**(12)**recorded12 (4.2%) persons in the 0-9 age group were infected with this pathogen, in addition **(4)** mentioned thatthe highest frequency of diarrheal diseases in the Federal Capital Territory, Abuja occurs within the age group of zero to five years, and this result in agreement with our results as shown in Tables (4,5,6).

Table (4): The prevalence level of diarreheal causative agents in humans stool samples according to gensder

Sex	isolated bacteria						
	No	Salmonella entritidis	% sal	E coli	%E.coli	E coli O157:H7	% E coli O157:H7
female	42	1	2.4	35	83.3	2	4.8
male	58	2	3.4	53	91.4	2	3.4
total	100	3		88		4	

Table (5):The prevalence level of diarrheal causative agents in dog stool samples according to sex

sex	isolated bacteria						
	No	Salmonella entritidis	% sal	E.coli	%E.coli	E.coli O157:H7	% E.coli O157:H7
female	49	1	2.0	42	85.7	1	2.0
male	51	1	2.0	44	86.3	3	5.9
total	100	2		86		4	

Table(6):The prevalence level of diarreheal causative agents in human stool samples according to Age

Age (year)	isolated bacteria						
	No	Salmonella entritidis	% sal	E coli	%E.coli	E coli O157:H7	% E coli O157:H7
<1	6	0	0.0	6	100.0	0	0.0
1_4	22	2	9.1	17	77.3	2	9.1
5_14	32	1	3.1	32	100.0	2	6.3
15_45	39	0	0.0	30	76.9	0	0.0
>45	4	0	0.0	4	100.0	0	0.0
total	103	3		89		4	

Genotyping

PCR result of *E. coli* O157:H7

The PCR assay was used to confirm the results of Chrom agar, PCR assay showed that all 8 isolates expressed (rfbO157) gene, 4 human stool isolates and 4 dog fecal isolates, also 4 isolates of human stool isolates showed (flicH7) gene, and 4 isolates from dog fecal isolates showed (flicH7), (Fig: 4).(Fig: 5)

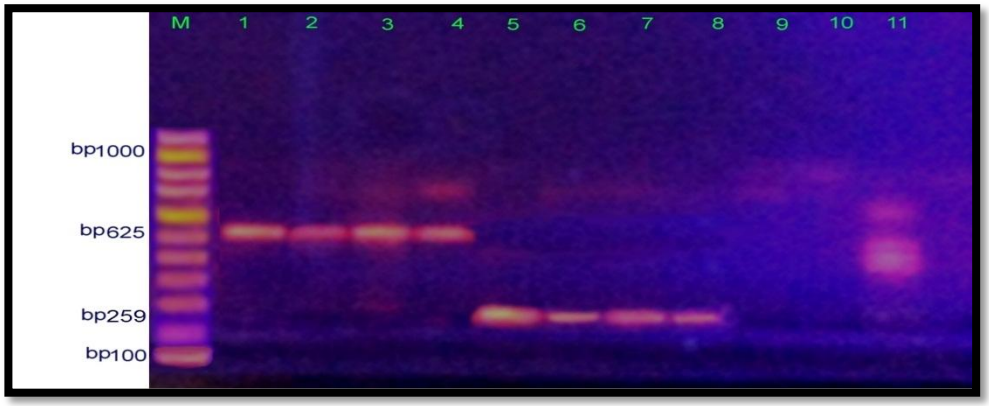
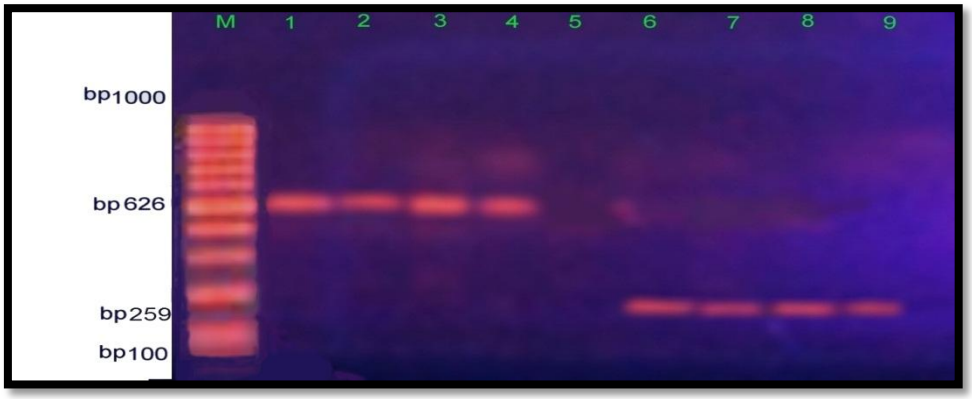


Figure (4): Agarose gel (2%) electrophoresis shows amplification of 259 bp& 625 bp fragments of (rfbO157) and (flicH7) genes by multiplex PCR. Lanes: 1, 2, 3, 4, 5, 6, 7, and 8 positive amplification of *E. coli* O157:H7 for human. Lane M: 100 bp DNA marker.

Four isolates of *E. coli* O157:H7from each origin E.coli out of (88) human stool isolates and (86) dog stool isolates of *E. coli*. these results agreed with .**{23}**, who reported that PCR assays are proven specific and sensitive in detecting microbial pathogens such as *E. coli* 0157:H7. Also **{20}**mentioned that gene based method such as PCR technique is more reliable than biochemical and serological tests for diagnosis of *E. coli* O157:H7. The main advantage of the employed PCR method is its ability to detect rough isolates or the isolates having a masked O antigen **{9}.**



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IV. Conclusion

The study concluded that there is a genetic relationship between human and dog's isolates, which was confirmed by conventional biochemical tests and PCR amplification targeting the ***flicH7, rfbO157*** of*E.coli O157:H7*.

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