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Detection Of Pathogenic Escherichia Coli O157:H7 From Euphrates River And Sheep In AL-Fallujah With Detection Of The Important Virulence Factor Rfb O157 And Flic H7 Genes Which Cause Haemorrhaic Diarrhea In Sheep

Muna M. Alkarbolii* and Mohanad Falhi Hamood
Public Health Department College of Veterinary Medicine University of Baghdad, Iraq
*Email: Munaalkarbolii@gmail.com , 0009-0003-6793-5038

Abstract
*The aim of the present study to isolation and identification the certain Escherichia coli associated with diarrhea sample of sheep which cause hemorrhagic diarrhea and detection of the important virulence factor Rfb O157 and fliC H7 genes in E.coli which cause diarrhea in addition to determine the antibiotic resistance of these bacteria. To achieve the goal, 50 of (diarrhea) samples were collected from sheep and 50 sample from water of river using routine bacteriological isolation and identification. The result showed that among 50 small ruminant (sheep) ,31% expressed bacterial isolates,the result of water from Euphrates River in AL- Fallujah (50 sample) was 27 positive isolate which equal **(54%)**. A statistical analysis of latex agglutination test revealed no significant differences in the prevalence of E. coli O157:H7 between sources ,Which revealed the E. coli O157:H7 in latex agglutination test produced positive isolation rates The O157 somatic antigen was found in 5(16.1)% and 6(22.2%) of E. coli isolates from sheep and water respectively and isolates flagellar H7 antigen was evaluated. The frequency of H7 antigen-positive isolates was 2(6.4%) for sheep isolates, and 1(3.7%) for water isolates. In addition to determine the antibiotic resistance of these bacteria These bacteria showed multi antibiotic resistance which resist for seven antibiotic agents and sensitive for three others were use. The result revealed that all **E.coli** isolates expressed rfbo157 gene, and showed positive result for fliCH7 gene. from **E.coli O157:H7** species pathogenicity and toxin production detection was done on 20 positive isolates from human, sheep and water samples were confirmed. Toxin is exclusively Enterotoxin (lipopolysaccharide endotoxin) was produced by E. coli isolates from human and sheep fecal samples, as well as water samples. ability to produce two types of virulence factors adhesins that promote binding to specific enterocyte receptors for intestinal colonization and enterotoxins responsible for fluid secretion. survival pathogen due to its antiphagocytic activity and confirm diagnosis of Rfb O157 and fliC H7 gene by PCR assay and gene sequence assay as well as determine antibiotic ability of Escherichia coli.*

***Keywords*: Escherichia coli, diarrhea ,hemorrhagic ,genes**

INTRODUCTION

Water is essential for sustaining life, but a significant proportion of the population lacks access to uncontaminated and secures potable water, resulting in numerous fatalities due to waterborne bacterial illnesses including cholera, typhoid fever, and bacillary dysentery **(41)**. *E. coli* of all irrigation water was more than the FDA limit, which indicated the fecal contamination **(8).**Acute microbial diarrheal illnesses pose a significant public health threat in economically disadvantaged nations. Diarrheal infections have a greater impact on those who are economically disadvantaged and reside in regions with inadequate sanitation. Young children, particularly in African and Asian countries, are more vulnerable to waterborne infections caused by microbes. As stated by **(85)** *Escherichia coli* commonly colonizes human infants' gastrointestinal tracts within a few hours after birth **(3).**Escherichia coli, Citrobacter, Klebsiella, Proteus, and Enterobacter are the main genera of the Enterobacteriaceae family. Most persons often harbor Citrobacter and Klebsiella bacteria, but in smaller quantities. Proteus and Enterobacter are found in a small minority of individuals **(97)** *Escherichia coli (E. coli)* is commonly found in the intestinal flora of both animals and humans **(1).** *E. coli* microorganism can cause many serious clinical symptoms such as bloody diarrhea, fever, and hemolytic uremic syndrome **(43).(6)** Escherichia coli, often referred to as E. coli, is a significant contributor to the digestive tracts of all organisms. Certain strains of E. coli, however, possess the capacity to be dangerous and induce sickness **(20)**. Escherichia coli (E. coli) has emerged as a

resilient pathogen, showing resistance to multiple antibiotics **(57).** Typically, human or animal excrement-polluted water poses the main hazards linked to microorganisms. The release of wastewater into both freshwater and coastal seawater is the primary reason for the existence of fecal bacteria, which might result in the transmission of diseases. **(95)**, **(35)**. *E. coli* O157:H7 may be introduced into the soil through irrigation water contaminated with cattle feces or through contact with contaminated surface runoff from the dairy farm **(63)**. Bacterial species is responsible for more than 95% of urinary tract infections, the organism that causes acute infections most frequently is *E. coli* **(7).**

MATERIALS AND METHODS

Samples collection

Isolation of *E. coli* was done with level 2 biosafety measurements and done on three stages , 50 sample form Sheep (collect stool sheeps' sample(diarrhea stool) from veterinarian hospital in al-anbar) and 50 sample from water of Euphrates river in AL-falluja by polyethelen tube which were performed from (January/ 2024-April/2024) for all samples of sheep and water.

Bacterial isolation and identification

The study included the Isolation of *E. coli* , the number of bacterial isolation species which were 100 sample , all water samples were stored at 4°C and used within 1 week of collection **(65),** then the sample was collected with a clean, detergent-free disposable screw-capped bottle, and labeled with the patient's code number. On-spot gross examination of diarrhea samples was performed to note the type of diarrhea. Then, about 2 gm of each fecal sample was added to culture tubes containing 10ml tryptone soya broth (TSB) for transportation to the laboratory in a cooler box within 2 hours of collection inoculated into media to cultured aerobically for 24 hours on blood agar , MacConkey agar and nutrient agar then put in incubation at 37 c^0 after check the incubation free from contamination by put un-inoculated plate of BHI agar for 24hours before put the isolated in the next day the bacterial growth was purified on selective media . **(19)**. Thereafter, a pure colony from each isolate was treated by staining with Gram's stain to determine the shape, arrangement and Gram's reaction of the bacteria. After that, a pure colonies were subculture distribute on different selective media and biochemical tests for identification of the bacterial isolates using traditional diagnostic methods. The conventional diagnostic tests used included: catalase, oxidase, hemolysis on blood agar, urease production, mannitol fermentation. moreover, motility, citrate utilization, Voges-proskauer, Indole production, methyl red, kligler iron test and H2S production, and used Anaerobic culturing was performed for some strain. Furthermore Diagnostic S.R.O were used and some suspected isolates were confirmed by Vitek® assay.

Antibacterial susceptibility test

Four to five freshly cultivated colonies of STEC O157:H7 were suspended in three to five milliliters of s terile physiological saline, with turbidity adjusted to a 0.5 McFarland standard, to create bacterial inocul ate**(36).**The whole surface of the MullerHinton agar was uniformly inoculated with a sterile cotton swab after being immersed, rotated multiple times, and pressed against the test tube wall.Sterile thumb force ps were utilized to position antibioticimpregnated disks on the surface of the inoculation plates followin g their dedication.The plates were incubated for twenty-four hours at 37°C under aerobic conditions.
A transparent ruler was placed above the plates to measure and document the diametr of the inhibition zone encircling each disk on the dark surface.The antibiotic susceptibility profile was categorized as susc eptible (S), intermediate (1), and resistant (R) according to CLSI recommendations. **(78) ; (81).** Furthermore, profiles of Multiple Drug Resistance, defined as resistance to a minimum of three classes of antibiotics, were established against the frequently utilized classes of antimicrobials.Cephem class (cef triaxone, ceftazidime, cefixime, cefuroxime), βlactam combination class (amoxicillin/clavulanate), Amin oglycoside class (gentamicin), Fluoroquinolone class (ciprofloxacin, norfloxacin), Tetracycline class (tetra cycline), Folate pathway inhibitors (sulfamethoxazole/trimethoprim), Phenicol class (chloramphenicol). **(22)**

Serological identification of E. coli O157:H7

The RIMTM E. coli O157:H7 latex test (R24250, Oxoid) was employed for serological confirmation fol lowing cultivation on MacConkey agars (CT-SMAC).

Three reagents are utilized in RIMTM E. coli O157:H7 latex.The particles of each reagent have been co ated with distinct antibodies: one targeting STEC serotype O157, another for STEC serotype H7, and a third utilizing normal rabbit globulin as a control substance.A droplet of latex was applied to the circle on the reaction card.A loop was employed to collect ten distinct colonies, which were subsequently intr oduced into the circular vessel containing the latex reagent.Recent colonies of STEC O157:H7 were co mbined with the test latex particles, as confirmed by CT-SMAC.Colonies exhibiting agglutination within one minute were classified as positive for STEC O157: H7.The test isolates were not STEC O157:H7, as evidenced by the lack of agglutination.
The control Latex reagent identifies non-specific reactions. **(77).**

RESULTS AND DISCUSSION

Isolation from *E. coli O157:H7*

The result of ***E.coli*** species isolation showed among one hundred sample divided as: 50 diarrheal sample of sheep from Veterinary hospital in Al-Anbar the result of ***E.coli*** species was 31 positive isolate which equal **(62%)** and the result of water from Euphrates River in AL- Fallujah (50 sample) was 27 positive isolate which equal **(54%)** , which reveal in table (1).

Source of sample	**Number of samples**	**Positive isolation of *E.Coli* species**	**Percentage % of positive no.**
Sheep	50	31	**62%**
Water	50	27	**54 %**
Total	100	58	**58 %**

table (1). The number of samples collected from sheep and water with positive isolates of ***E.Coli*** species and percentage % of them.as well as **(44)** Found that A total of 340 samples was collected: 45 from milk, 45 from sheep drinking water, 125 from the feces of healthy sheep, and 125 from the stools of diarrheal patients. Non-sorbitol fermenting colonies (NSF) were identified in the samples utilizing sorbitol MacConkey agar augmented with potassium tellurite and cefixime (CT-SMAC). Of the 340 samples, 58 (46.4%) were identified as diarrheal stool, 98 (78.4%) as sheep dung, 19 (42.2%) as milk, and 17 (37.0%) as drinking water. Three (5.7%) isolations from stool samples of diarrhea patients, this agreement with our study were record the result of *E.coli* species isolation showed among one hundred sample divided as: diarrhea samples from sheep (50 sample), the result of *E.coli* species was 31 positive isolate which equal (62%) and the result of water from Euphrates River in AL- Fallujah (50 sample) was 27 positive isolate which equal (54%) These result aggree with **(50 ; 24).**The current study revealed bacterial isolates from water, animal and human, in addition all these isolates are virulent bacteria, these results may indicate that pathogen may play a role in diarrhea of human and animal these ideas are in agreement with **(44)** who recorded that e coli o157:H7 are form most diarrhea infections, also the present finding may indicate that these pathogens cause small proportion of individuals may develop hemolytic-uremic syndrome (HUS) after diarrhea. The occurrence of this life-threatening complication is linked to substantial illness in those who survive, **(17); (50).**The current study showed that Escherichia. coli 30 16.8 high percentage of bacterial isolates from water these results may give indication that these pathogens play a role in diarrhea infection these results are in agreement with **(38)**.Who revealed that the E.coli form the most bacterial isolates from waterThe isolation of bacteria from sheep samples may indicate that these bacteria can reach the blood stream from enters the colonic epithelial cell, breaks down the phagosome, and moves into the cell via creating actin microfilaments. to spread horizontally across the epithelium, the bacteria may either exit and re-enter the baso-lateral plasma membrane or directly move from one cell to another **(39).** Our result agreement with **(64)** who record bacterial isolates from collected fecal samples of diarrheic sheep found that *E. coli* was present in 16 samples (32.00%), *Salmonella* in nine samples (18.00%), *Enterobacter* spp. in five samples (10.00%), *proteus* in four samples (8.00%), *Citrobacter* spp. in

four samples (8.00%), *Klebsiella* spp. in four samples (8.00%), *Providencia* spp. in three samples (6.00%), *Serrattia* spp. in two samples (4.00%) and about three samples (6.00%) were mixed infections. **(92)** record this result who isolated E. coli from fecal samples of diarrheic sheep Our results are agreement with **(66)** who isolated *E. coli* (34.20 %), *Salmonella* (5.26%), *Proteus* (13.10%), *Klebsiella* (7.89%) and mixed infection (21.00%). In the current study *E. coli, which* causes bacterial diarrhea in sheep, several investigations are isolated of E. coli with various percentages. Sweeny et al. (2012). The prevalence of *E. coli* in the current study was agreed with the findings of **(17).** The differences of the prevalence rates of *E. coli* in diarrheic lambs and sheep may be attributed also to the geographical locations and management practice as well as hygienic measures where ETEC infection occurs mainly through ingestion of contaminated food or water record by **(89).**In current study **(5)** who found Strains isolated from the feces of slaughtered ruminants, including camels, cattle, sheep, and goats, in the United Arab Emirates were analyzed for the presence of the E. coli O157 serotype, its key virulence genes, and related antibiotic resistance. which agree with our study when isolated from the feces of ruminants In particular sheep

Characterization of *E. coli* strains

E. coli under microscope in Gram stain showing gram-negative rods shape , **on nutrient agar appear large, moist, thick, yellowish white to greyish white Culture of *E.Coli* ,Characteristics on MacConkey agar as** circular, moist Colonies appear pink and lactose fermenting with mucoid texture **Culture of *E. coli* characteristics on** Eosin Methylene Blue (EMB) Agar Colonies appear Green Metallic sheen **and on** Cystine Lactose Electrolyte-Deficient (CLED) Agar Colonies appear yellow and lactose-positive and on Chromogenic Agar media appear blue-green with dark center with clear beige color of media **Culture of *E.coli O157:H7* strain are identification**

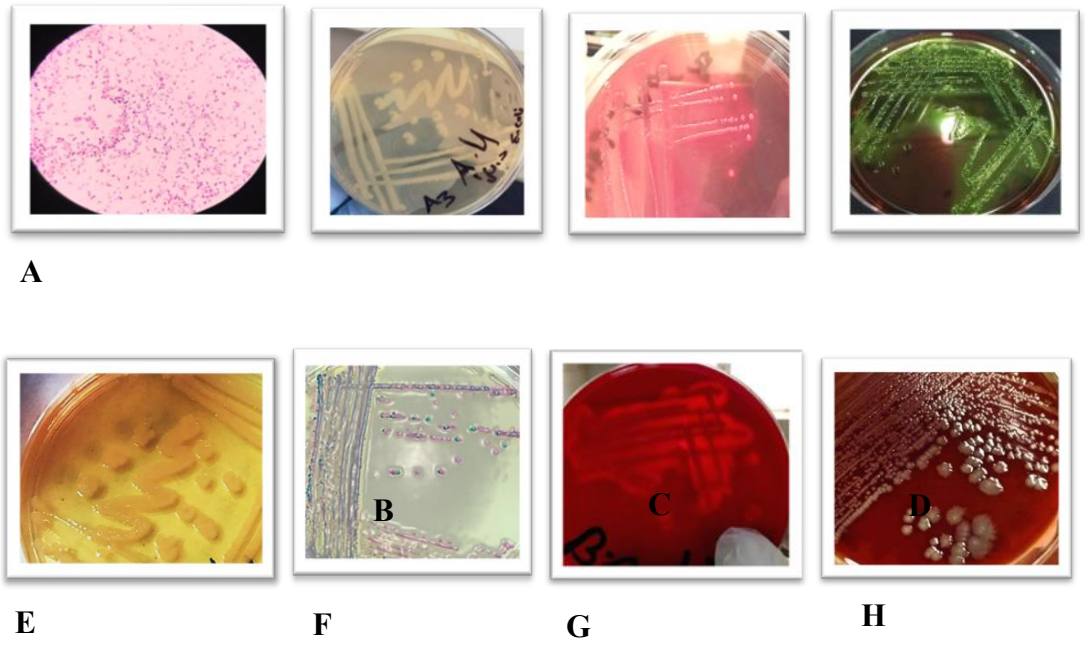

Figure (1) ***A)E. coli O157:H7*** under microscope in Gram stain showing gram-negative rods shape ***B) E. coli* on nutrient agar appear large, moist, thick, yellowish white to greyish white Culture of *E.Coli O157:H7* characteristics** C) **on MacConkey agar as** circular, moist Colonies appear pink and lactose fermenting with mucoid texture **Culture of** D) ***E. coli O157:H7* characteristics on** Eosin Methylene Blue (EMB) Agar Colonies appear Green Metallic sheen, E) **on** Cystine Lactose Electrolyte-Deficient (CLED) Agar Colonies appear yellow and lactose-positive ,F) on Chromogenic Agar media appear blue-green with dark center with clear beige color of media)G) E.coli on sheep blood agar appear of β- hemolysis , H) **Culture of *E.coli O157:H7* characteristics on** Blood Agar Colonies appear big, circular ,moist , gray to whiteish color.*E.coli* on sheep blood agar appear of β- hemolysis , **Culture of *E.coli O157:H7* characteristics on** Blood Agar Colonies appear big, circular ,moist , gray to whiteish color.The current

results It is characterized by being gram-negative, having a straight and rod-shaped structure, and lacking spores these idea are in agreement with **(16)**, these characteristics are in agreement with those mentioned in **(71) and (87).** showed that all the isolates were distinguished by their short straight rods shape, with single or bilateral groupings, and were negative for cream pigment, pinkish-reddish color.**(29)** record that Nutrient agar, Mac Conkey agar, and EMB agar are often used culture medium for producing E. coli due to the bacteria's fundamental nutritional requirements, the optimal temperature for E. coli growth is 37°C (98°F). Under optimal conditions, the process of reproduction may be accomplished within a few twenty minutes **(18)**. These results are in agreement with Surinder K. (2012) states that E. coli generates pink colonies on Mac Conkey agar due to lactose fermentation and large, thick, greyish-white, moist, smooth, opaque, or translucent disc-like colonies on Nutrient agar. Lactose fermentation, in contrast, alters the medium's pH to an acidic level, corroborating the findings of (10)On Eosin methylene blue agar, E. coli exhibits a green metallic sheen due to metachromatic pigmen ts.Isolation of E. coli strains, a loopful from the MacConkey broth was inoculated into MacConkey's agar and incubated at 37.00 ˚C for 24 hr. Lactose fermenter (pink) colonies were streaked onto and Eosin Methylene Blue agar and confirmed as E. coli using the standard biochemical tests according to (23).The enriched samples were inoculated onto MacConkey agar and incubated aerobically for24 hours at 37°C.The feature and reactivity of the isolates on agar media were reveal and documented post-incubation Five to ten pinkishhued, dubious colonies were subculture onto an alternative nutritional agar (73)(76)indicated that Escherichia coli cultivated on cystine lactose electrolyte deficient agar exhibits blu e colonies for nonlactose fermenters and yellow colonies for lactose fermenters.Our data corroborates t heir conclusions. These result agreement with **(42)** who record that medium was formulated by replacing lactose with sorbose and raffinose and replacing neutral red with phenol red and bromothymol blue. The formulated medium has a clear beige color. **(83)** who Found that, On sheep blood agar plates from fresh colonies of PCR positive isolate and incubated at 37°C for 24-hour, after 8h plate appear hemolysis (α hemolysis) then develop to clear zones of β- hemolysis around the colonies after 24h, and that agreement with **(54). (54)** indicated that E. coli O157:H7 colonies exhibit large, spherical, moist, gray to whitish pigmentation on Blood Agar, corroborating our findings. In the current study,our result agreement with **(90)** who record that all strains were confirmed as *E. coli* with an API 20E kit (bioMerieux) .In the current study, **(49)** who found a perfact resutt for his research by using s.r.o. kite for diagnosis . The result of biochemical test of ***E.Coli*** are Motility + ,Oxidase -,Catalase+ Simmon Citrate utilization - ,Indole +,Urea hydrolysis+ ,Methyl red + ,Voges Proskauer + ,H2S Production -, A/A*TSI Gas formation ,Gelatin hydrolysis – In the current study,our result agreement with **(93)** who record that the Indole test had ability of bacteria to produce indole from tryptophan and find out the ability of isolates to ferment glucose and produce acids in Methyl red test to Changing the color of the media to red and Simmon's citrate test are green that agreement with **(89).** who record appearance of bubbles is considered positive in Catalase test **(47)** who record that many types of acids, including lactic, acetic, and formic acids. These acids may be detected by introducing a pH-sensitive indicator, such as phenol red or methyl red. Furthermore, *E. coli* has catalase activity and lacks oxidase activity, in addition to its ability to reduce nitrates. *E. coli* may be verified by a series of further biochemical tests. Voges and Proskauer devised a test to detect acetoin and 2,3-butanediol. The samples (rectal swabs) were cultivated aerobically then bacterial isolates use for studying biochemical characteristics according to **(74).**These result agreements with **(48)** who record Standard bio-chemical tests for detection of *E. coli* were performed for 16 positive isolates including indole production test, methyl red test, nitrite reduction, ONPG, Sugar fermentation as lactose and arabinose. In current study **(19),** who record Positive Indole test results, negative Simon's citrate agar and urease test outcomes, together with lactose and glucose fermentation shown by Triple Sugar Iron (TSI) exhibiting acid generation and absence of hydrogen sulfide (H2S), are biochemical characteristics characteristic of E. coli. These result agreements with our study which the same result of biochemical test include Positive Indole test, negative Simon's citrate agar and urease test, lactose and glucose fermentation shown by (TSI) exhibiting acid generation and absence of (H2S), these agreement with **(79)**.

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Identification by using API 20 E , Vitek2 system and using DIAGNOSTICS s.r.o. kite (GN24).
E. coli isolates was identified by using of API 20 E to Identification throughout choose isolate were sheep and water to detect the species.

Figure (2) ***E. coli*** isolates was identified by using of the API 20E.

Vitek2 system confirmed the diagnosis of some suspected *E.coli* with 99% probability value, based on the results of 64 biochemical tests

Organism Origin			VITEK 2																
Selected Organism			99% Probability			Escherichia coli													
			Bionumber: 0405610450006600										Confidence:			Excellent identification			
SRF Organism																			
Analysis Organisms and Tests to Separate:																			
Analysis Messages:																			
Contraindicating Typical Biopattern(s)																			
Biochemical Details																			
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+		
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+		
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-		
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+		
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-		
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+		
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-					

Fig (3) Vitek2 system results that confirmed *E. coli* with 99% probability value

E. coli isolates was identified using DIAGNOSTICS s.r.o. kite (GN24) gram negative for biochemical identification. The results are identified by software inputting additional tests enter results of individual tests isolates was identified 100% with 1.00 excellent differentiation.

ID No.	28	Batch No.	12	Shelf life	30
evaluated by	muna maher	Lab	college	Sample	stool
Diagnosis	diarrhea	Comment			

Taxa	Identification (%)	Differentiation (T)
Escherichia coli	excellent 100.00	excellent 1.00

OXI		URE	GLU	H2S	ARG	ORN	LYS	SCI	bGL	PHE
-		-	+	-	-	o	+	-	-	-
OK		OK	OK	OK	OK		OK	OK	OK	OK
	IND	NAG	SUC	TRE	MAN	LAC	CEL	MAL	GGT	PHS
	+	o	o	+	+	+	-	-	-	o
	OK			OK	OK	OK	OK	OK	OK	
	GLR	ESL	DUL	ADO	SOR	RHA	RAF	INO	bGA	NIT
	+	o	o	-	+	+	o	-	+	+
	OK			OK	OK	OK		OK	OK	OK
		VP	PYR	ONP	G42	bHEM	YEP			
		-	-	+	-	+	-			
		OK	OK	OK	OK	OK	OK			

Figure (4) ***E. coli*** isolates was identified 100% with 1.00 excellent differentiation by using

DIAGNOSTICS s.r.o. kite **(68)** which corresponds to the presence of both commensal and pathogenic *E. coli*. **(9) and (27)** *found* the ability of pathogenic *E. coli* to survive the chlorination and UV stages of wastewater Vitek 2 compact system in monitoring wastewater samples for the presence of antimicrobial

resistance *E. coil.* This method is not only confined to the detection of *E. coil* but can be used in the identification of other waterborne pathogens including *Salmonella* and *Vibrio cholerae* in wastewater samples. The biochemical tests, vitek®2 were used to confirm positive samples, to correctly identify the agent of infection needed VITEK®2 in the medical field because its Quick and precise methods. to diagnosis of infectious pathogen such *E.coli* we need this Automated systems **(62).**

In current study, (30) confirmed *E. coli* by API 20E identification system (Biomerieux MarcyI'Etoile, France). the isolates were preserved at −70 °C which cultured on tryptic soy broth has twenty percentage sterile glycerol,this agreement with our study when confirmed *E. coli* by Vitek2 system results with 99% probability value.

Serological identification of E. coli O157:H7

The quality of the somatic O157 antigen was assessed using a latex agglutination test. The O157 somatic antigen was found in 5(16.1)% and 6(22.2%) of E. coli isolates from sheep and water respectively, according to the results. The O157 result that was positive.

The isolates flagellar H7 antigen was evaluated. The frequency of H7 antigen-positive isolates was 2 (6.4%) for sheep isolates, and 1(3.7%) for water isolates .A statistical analysis revealed no significant differences in the prevalence of E. coli O157:H7 between sources show in (Table 3).

Isolates Source	*E. coli* Isolates	O157 Positive	H7 positive
Sheep	31	5(16.1%)	2(6.4%)
Water	27	6(22.2%)	1(3.7%)
	X2= 0.986 p>0.05		X2=4.836 p>0.05

(Table 3). Show A statistical analysis revealed no significant differences in the prevalence of E. coli O157:H7 between sources The current investigation **(44)** revealed that the E. coli O157:H7 latex agglutination test produced positive isolation rates of results revealed that 11.5%, 11.5%, 16.6%, and 23% of E. coli isolates from human, sheep, milk, and water respectively were positive for O157 somatic antigen. The O157 positive isolates tested for the flagellar H7 antigen. Frequency of H7 antigen positive isolates were 5.7%, 7.3%, 11.1%, and 0% for human, sheep, milk, and water isolates respectively ,this agreement with our study when the quality of the somatic O157 antigen was assessed using a latex agglutination test. The O157 somatic antigen was found in 5(16.1)% and 6(22.2%) of E. coli isolates from sheep and water respectively, according to the results. The O157 result that was positive ,the isolates flagellar H7 antigen was evaluated. The frequency of H7 antigen-positive isolates was 2(6.4%) for sheep isolates, and 1(3.7%) for water isolates. **(60)** who found appearance of granulation is an indication that the isolate possesses the O157 antigen and H7antigen;but the absence of granulation indicates that the isolate does not possess this antigen. **(98)** explained that the latex test is one of the most important rapid confirmatory tests for the serotype of *E. coli* O157: H7, which is easy to use because it requires less time and effort. **(75)** indicated that this method is one of the traditional methods of detecting this pattern. **(58)** that record Quality detection of somatic O157 antigen was done by latex agglutination test . The results revealed that 11.5%, 11.5%, 16.6%, and 23% of E. coli isolates from human, sheep, milk, and water respectively were positive for O157 somatic antigen.these agreement with our result which human, sheep, and water respectively were12.9% ,16.1% ,22.2% . Statistical analysis revealed no significant differences in the frequency of E. coli O157:H7 from different sources. **(90)** who found that as serotype O157 with a latex agglutination kit (Oxoid) in this current study correspond with our result.These result agreement with **(46)** using rapid diagnostic *E. coli* antisera sets for diagnosis . **(44)** record that seven (7.3%) isolates from sheep feces, two (11.1%) isolates from milk, and no positive results from drinking water samples were identified as E. coli O157:H7 by a latex agglutination test. These idea agreement with our result were isolates from sheep feces 31 which O157 Positive were 5 (16.1%) & H7 positive were 2(6.4%) , but; Disaggrement with results from drinking water samples were identified as E. coli O157:H7 because our result were 27 number of E. coli O157:H7 isolaton which O157 Positive were 6 (22.2%) & H7 positive were 1 (3.7%) this idea are in agreement with observation of **(12).** In the present investigation

(44) record that, no E. coli O157:H7 was isolated from water samples using the PCR method or the latex agglutination test. However, these idea agree with **(51)** reported that 1.3% of E. coli O157:H7 was identified from water troughs on farms in Washington, Oregon, and Idaho.

Antibacterial susceptibility test

E. coli O157:H7 isolates was performed on Muller-Hinton agar plate (Oxoid) for antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method following the results were explained according to Clinical and Laboratory Standards Institute-**(22)** This test was done for 10 isolates of 10 antibacterial agents. the table **(4)** revealed the number of sensitivities, Resistant and intermediate of *E. coli* to certain antibiotic.

Antibiotic agent	**Disk content Microgram**	**Inhibition zone (mm)**	**I***	**S***	**% of 10 antibiotic agent S* to 10 samples**	**R***	**% of 10 antibiotic agent R* to 10 samples**
Ciprofloxacin	5	33.80	16-20	≤15	20	≥21	50
Levofloxacin	5	18.30	14-16	≤13	60	≥17	50
amoxicillin/clavulanate	20/10	18.94	13-14	≤12	30	≥15	40
Cefepime	30	14.40	15-17	≤14	30	≥18	60
Tetracycline	30	23.13	15-16	≤14	20	≥17	70
Ofloxacin	5	22.39	13-15	≤12	20	≥16	50
Chloramphenicol	30	13.61			40		60
Impenem	10	22.97	16-18	≤15	50	≥19	40
Gentamycin	10	21.60	16-21	≤15	50		40
Piperacillin+tazobactam	100/10	27.74	15-20	≤14	20	≥21	60

Table (4) reveals the number of sensitivities, Resistant and intermediate of *E. coli* to certain antibiotic

In current study,**(52).** Who record, in United States of America. the result can be attributed to several factors, chief among them being that all the troughs in the investigation that yielded positive results for E. coli O157:H7 were situated in a shaded region away from direct sunlight. Direct sunlight decreases the survival of E. coli in aquatic conditions **(14)**. However, it is typical for the concentration of bacteria in sediments to be up to 1000 times higher than that in the water column above them **(11)**. Consequently, the isolation rate makes the sample methods used in our study (surface water) and the other study very evident. Competition with other aquatic species affects the survival of E. coli because the animals in this study got their water straight from the river **(34); (59)**. A common pathogen such as *Escherichia coli* is related with community-associated with nosocomial infections **(70)**; **(26) ,** but; at last few years, the emergence and broad dissemination of *E. coli* strains revealing resistance to broad-spectrum of

antimicrobial agents which reported **(70); (15); (69)** , these agreement with our study which show resistance of *E.coli* to multiple antimicrobial agents revel that in laboratory when performed disk diffusion techniques and cultured which isolate were from diarrhea samples of human and sheep with samples from water has multidrug resistant which cause threat a public health,This idea agreement with **(15)** and **(69)** whom record Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has a significant public health impendence as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria.*E. coli* strain reveal expressed resistant to most antibiotic examination which using in our present study, the result of this strains which isolated from diarrhea samples of human and sheep with samples from water characterized by multidrug resistant, these results which supported the idea of **(39)** who record that *Escherichia coli* were Multidrug-resistant has become a major public health in many countries, leading to failure in treatment with consequent wide health burden, the resistance rates *were* trimethoprim-sulfamethoxazole 88.3%, tetracycline 77.1%, ciprofloxacin 58.4%, ofloxacin 55.1%, amoxicillin-clavulanate 50.4%, ceftazidime, gentamicin 35% each, chloramphenicol, which agreement with our study for the same antibiotic resistance with high level were Ciprofloxacin,Levofloxacin, amoxicillin/clavulanate ,Cefepime, ,tetracycline,Ofloxacin ,chloramphenicol , ,Gentamycin ,Piperacillin+tazobactam, Impenem as the following 50% ,50%, 60%, 70% ,50% ,40% 60%,40%, 60%,40% respectively. That approved by **(21)** whom found that the *E. coli* characterized by multiantibiotic resistance. **(55)** who record the result of resistance and intermediate were gazed as non-susceptible Multiantibiotic resistant of *E. coli* was defined as non-susceptibility to fully one agent in three or more antimicrobial categories which agreement with our study that reveal multiantibiotic resistant and intermediate result of E. coli against many antibiotic agents. In current study **, (2)** who record antimicrobial sensitivity tests were performed on patients with diarrhea or urinary tract infections, Escherichia coli showed high resistance rates of antibiotic resistance against antimicrobial agents such as ampicillin, amoxicillin, chloramphenicol, tetracycline, cotrimoxazole, nalidixic acid, sulfonamide, and neomycin, and were completely sensitive to ciprofloxacin ,which agreement with **(39)** who record the predominant pattern of resistance was observed against ampicilln, amxicillin, tetracycline, cotrimoxazole, and sulfonamid But; these study disaggrement with our study which Ciprofloxacin,Levofloxacin, amoxicillin/clavulanate ,Cefepime, ,tetracycline,Ofloxacin ,chloramphenicol ,Gentamycin ,Piperacillin+tazobactam, Impenem ,which multiantibiotic resistant of E. coli to these antibiotic agents are not for ampicillin, amoxicillin, cotrimoxazole, tetracycline, sulfonamide, trimethoprim, streptomycin, and carbenicillin , but; our study agreement with **(28).**

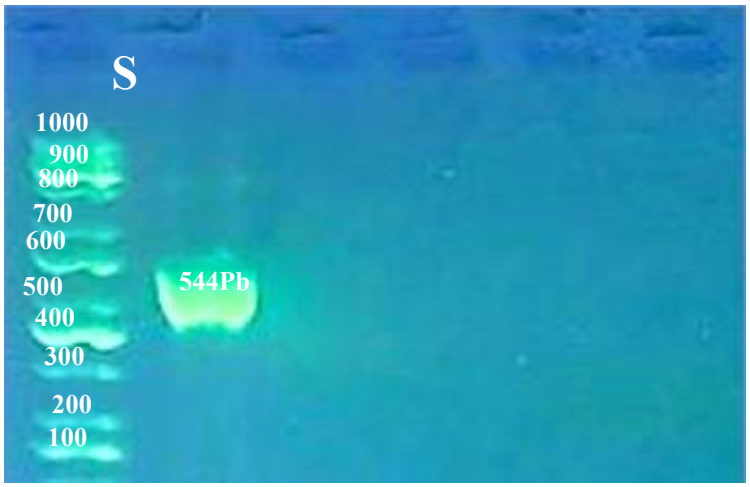
In the current study, our result agreement with **(29)** who record presence of antimicrobial resistance E. coil. In the sample waterwaste *E. coli* isolates showed high rates of resistance to erythromycin, amoxicillin and tetracycline. Nitrofurantoin, norflaxocin, gentamicin and ciprofloxacin are considered appropriate for empirical treatment of *E. coli* in the study area. Regular monitoring of antimicrobial susceptibility is recommended. **(45)**. In the current study **(92)** who record multiple Drug Resistance, resistance to at least three antibiotic classes, profiles were determined against the commonly used classes of antimicrobials, Cephem class (ceftriaxone, ceftazidime, cefixime, cefuroxime), β-lactam combination class (amoxicillin/clavulanate), Aminoglycoside class (gentamycin), Fluoroquinolone class (ciprofloxacin, norfloxacin), Tetracycline class (tetracycline), Folate pathway inhibitors (sulfamethoxazole/trimethoprim), Phenicols class (chloramphenicol).

MOLECULAR BIOLOGY STUDIES.

4.14.1. Molecular identification of *E. coli* isolates

From the same confirmed isolate, choose virulence a factor was found in each isolated sample of sheep and water the percentage were 100% from isolates 31 samples sheep and 27 sample from water) which found locally in Iraq , The results showed 100% identification of ***E. coli*** by PCR based on stander ladder bands in the figure (4.13.) as in table (4.5). In recent years, the significance of the PCR-based detection

technique for bacterial identification has increased. This is primarily justified by the fact that the DNA from a single bacterial cell may be amplified in around one hour when compared to previously reported procedures **(40); (97).** Twenty E. coli O157:H7 isolates were selected for the study, comprising positive samples from water, ovine sources, and humans. Enterohaemolysis production was evaluated in samples 10, 5, and 5 on sheep blood agar. All examined isolates exhibited positive β-hemolysis.and exhibit the function of enterohaemolysin fresh colonies of PCR-positive isolates were inoculated onto sheep blood



agar plates and incubated for 24 hours at 37 ± 1 °C, with plates exhibiting hemolysis being noted. after 24 hours, the target bacteria exhibit small, distinct β-hemolysis zones surrounding the colonies . In current study, **(44)** who record all (11)E. coli O157:H7 isolates were evaluated for enterohaemolysis production on sheep blood agar (SBA), all examined isolates exhibited positive β-hemolysis and demonstrated enterohemolysin activity through Identification of Enterohemolysin Synthesis. This result agreement with **(83) (82)** who record The enterohemolytic phenotype was restored upon reintroduction of a recombinant plasmid containing this determinant into the enterohemolysin-negative isogenic mutant and Following cloning, the hemolysin determinant plasmid of the strain was determined to be nearly identical to EHEC-hemolysin based on DNA and N-terminal amino acid sequence analysis. This agree with **(95)** when record study on bovine Shiga-like toxin-producing Escherichia coli (SLTEC) which produce the enterohemolysin phenotype and encoded by the EHEC-hemolysin gene. The amplification of the 16S rDNA gene segment was conducted using the F27 forward primer (5'- AGAGTTTGATCCTGGCTCAG -3') and the R1492 reverse primer (5'- CTTGTGCGGGGGCCCGTCAATTC -3'), with the results depicted in **(Figure 4.4)** indicating a comprehensive identification of *Escherichia coli*. The results support the utilization of this gene fragment, located at the 16S rRNA locus, as a specific identification marker for *Escherichia coli*. A strain was isolated from a water sample by **(56),** who subsequently characterized it using the sequences of the 16S ribosomal RNA gene and the 16S-23S rRNA internal transcribed spacer region. Escherichia coli was the closest phylogenetic relative, with 99% similarity in 16S rRNA gene sequences. The predominant primer for species-level identification is 27f/1492r **(32).** Through amplification and sequencing, presently accessible primers can elucidate the composition of the predominant microorganisms in a sample. This aligns with the findings of **(31)** with our research, wherein we utilized commonly employed primers to amplify the DNA spanning positions 27 to 1492 of bacterial 16S rRNA genes for Escherichia coli rRNA. The amplification of 16S rDNA in this work was conducted by **(56)** using two distinct approaches. The initial characteristic is PCR with universal primers 27F and 1492R, which encompass nearly the whole length of the 16S rRNA gene. This concept aligns with the findings of **(37)**, who employed gel-electrophoretic separation in denaturing gradients and selective amplification of genes encoding 16S rRNA to examine actinomycete communities**. (61); (33); (13).** Exhibited the significant effectiveness of 16S rRNA gene sequencing in bacterial categorization. **(72) and (87)** detail research designs utilizing PCR-based methodologies for micro diversity studies, specifically emphasizing universal-primed PCR of 16S rDNA in Escherichia coli. **(4)** who record Following the enumeration of positive samples from each animal in every collection, DNA was extracted and examined to ascertain the presence of virulent genes.

These are matches And agree with our method. The rate of PCR positive for E. coli O157:H7 isolates from human samples (20%) and that was higher than results obtained by Blanco [20] (3%).

4.2. Extraction and Detection of Rfb O157 and fliC H7 genes

The Polymerase Chain Reaction (PCR) method was employed to discover specific diagnostic genes (Rfb o157 and fliC H7), thereby confirming all E. coli isolates on EMB agar. **Figure (4.13).**

Pathogenicity and toxin production detection was done on 10 positive isolates from sheep and water samples confirmed E. *coli* O157:H7 isolates, by detecting the presence of rfbo157 gene (259bp) and *fliCH7* gene (625 bp) among all isolates were positive for rfbo157 gene, and showed positive result for *fliCH7* gene.

Source	No. of total sample	No. of total positive isolates	No. of chosen isolates for study	No. isolates carrying genes of virulence factors (***Rfb o157*** *gene and* ***fliC H7*** *gene*)	%
Sheep	50	31	5	5	16.1
Water	50	27	5	5	18.5

Table (4.5): percentage of prescence of virulence factors of *E. coli* in sheep and water isolates which carry genes of virulence factors (***Rfb o157*** *gene and* ***fliC H7*** *gene*) In current study, **(44)** who found *rfbO157* gene size (259 bp) There were 11.5%, 11.5%, 16.6%, and 23% positive isolation from human, sheep, milk, and water samples respectively, The *rfbO157* positive isolates then tested for the presence of *fliCH7* gene (625 bp). The results were 5.7%, 6.3%, 11.1%, and 0% positive isolates from human, sheep, milk, and water samples respectively . There were no significant differences in the frequency of *rfb*O157 and *fli*CH7 in the isolates from different sources. These idea agreement with our study which the *rfbO157* gene size (292bp) was estimated depending on DNA marker (100 bp DNA ladder). The result was 16.1 % and 18.5 % positive isolates fromsheep and water samples respectively including *rfbO157* positive isolates and *fliCH7* gene size (625 bp) was estimated depending on DNA marker (100 bp DNAladder) that reveal in (Table 4.5). The prevalences of rfbO157 and fliCH7 among the isolates were not statistically different. the same number and percentage in all isolates chosen for study which carrying genes of virulence factors (***Rfb o157*** *gene and* ***fliC H7*** *gene*) from different sources. To assess pathogenicity and toxin production, confirmed E. coli isolates were analyzed for the presence of the 292 bp rfbo157 gene. (Figure 4.13) illustrates that 20 isolates were selected from a total of 120, with 60% testing positive for this gene utilizing a 1000 bp DNA ladder. This study investigated the presence of the fliC H7 gene (625 bp) in verified E. coli isolates, with 20 isolates yielding positive results for this gene. as shown in table (4.6). these results are current with **(5). (4)** who record previously, to ascertain the existence of genes within the E. coli O157 antigen gene locus, rfbEO157:H7, which encodes GDP perosamine synthetase (rfbO157), uidA, and the H7 flagellar protein (fliCH7), multiplex PCR analysis was conducted on all biochemically confirmed E. coli isolates exhibiting O157 agglutination positivity. These matches with our study by following these steps methods. **(91)** conducted a recent investigation on the serotype E. coli O157:H7, revealing a significant prevalence of E. coli in salad samples, indicating fecal contamination. The rfbo157 gene indicates pathogenicity, while stx1 and stx2 signify the production of Shiga toxin. The pathogenicity of E. coli was established using molecular detection of the rfbo157 gene, corroborating our findings.

Gene names	**Sequences (5′.....3′)**	**Size**	**No. of chosen isolates for study**	**No. isolates carrying genes of virulence factors (*Rfb o157 gene and fliC H7 gene*)**	**Reference**
Forward *rfb o157*-F	GTGTCCATTTATACGGAC ATCCATG	292 base pair	10	10	**(Ningrum et al,.2016)** **(Imtiaz et al,. 2013)**
Reverse *rfb o157*-R	CCTATAACGTCATGCCAA TATTGCC	292 base pair	5	5	**(Ningrum et al,.2016)** **(Imtiaz et al,. 2013)**
Forward *fliC H7* F	GCGCTGTCGAGTTCTATC GAGC	625 base pair	5	5	**(Khudor et al,.2012)** **(Ningrum et al,.2016)**
Reverse *fliC H7* R	CAACGGTGACTTTATCGC CATTCC	625 base pair			**(Khudor et al,.2012)** **(Ningrum et al,.2016)**

Table (4.6): Sequencing of primers that are used to amplified *rfbo157 gene and fliC H7 gene*

CONCLUSIONS

Foodborn and waterborne pathogens include E. Coli isolates from sheep diarrheal sample and water samples , the E. Coli isolates in this study are pathogenic enterohaemorragic and pathogenic . ,the bacteria isolated from water resembled those seen in samples of sheep feces.,the predominant bacterial isolate identified in sheep and water the samples identification as E. coli O157:H7 strain. , Escherichia coli exhibited the capacity to acquire resistance to numerous antibiotics.,. A statistical analysis of latex agglutination test revealed no significant differences in the prevalence of E. coli O157:H7 between sources

produced positive isolation rates ,Enterotoxin (lipopolysaccharide endotoxin) was produced by E. coli isolates from human and sheep fecal samples, as well as water samples. ability to produce two types of virulence factors: adhesins that promote binding to specific enterocyte receptors for intestinal colonization and enterotoxins responsible for fluid secretion ,Molecular examination revealed The PCR analysis of human diarrhea sample 10, sheep diarrhea sample 5, and water revealed the presence of genes for the flagella H-antigen (fliC) and the surface O-antigen (rfb). The isolation demonstrated exceptional compatibility, exhibiting over 99% identity with the Neighbor-joining tree of the E. coli fliC and rfb genes.

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