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MOLECULAR IDENTIFICATION OF *ESCHERICHIA COLI SEROTYPE* O157:H7 FROM HUMANS AND CHICKENS IN IRAQ

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ABSTRACT

A total of 250 samples that involved fifty human stool Specimens and two hundred chicken feces which divided into (100 from broiler chickens swabs and 100 from laying chicken swabs) in the holy Karbala, after isolated and culture on several media, define their supposed virulence genes via PCR analysis. All the *E. coli* O157: H7 isolates confirm by PCR analysis by 16s rRNA, rfbO157 and fliCh7 genes, than study antibiotic susceptibility. Results 9 positive sample from human and chicken include 6%, 5% and 1% were respectively, from human, broiler and laying hens. Antibiotic susceptibility test from human showed that all isolated positive were susceptible 66.6% to (Gentamicin, Streptomycin and Chloramphenicol) low susceptible to other antibiotics, in contrast absolute resistant 100% against Ampicillin, Tetracycline and Amoxicillin 66.6%, Gentamicin, Neomycin, Chloramphenicol and Doxycycline 33.3% intermediate susceptible were reported in Neomycin, Streptomycin, Doxycycline 33.3%, the isolates *E. coli* O157:H7 from chicken showed that all isolated were susceptible 50% to(Gentamicin and Streptomycin) low susceptible to other antibiotics, in contrast absolute resistant 100% against (Ampicillin and Amoxicillin), Neomycin and Doxycycline 83.3%, Tetracycline 66.6%, Gentamicin, Chloramphenicol 50%, Streptomycin 33.3%, intermediate susceptible were reported in Chloramphenicol, Streptomycin 16.6 %.

Keywords: *E. coli* O157:H7, 16s rRNA gene, rfbO157 gene, fliCh7 gene, Antibiotic susceptibility

Introduction

E. coli can found in water, soil or other environments like gut of warm-blooded animals, add in man (WHO, 2016), *E. coli serotype* O157:H7 infections have continued to occur in great outbreaks and sporadic cases, while outbreaks were reduced after 1999 (Lim *et al.,* 2010). Virulent strains of *E. coli* cause colibacillosis, an important disease in poultry which also cause diarrheagenic episodes and deaths in humans (Abdela, 2017). *E. coli* serotype O157:H7 as a significant zoonotic pathogen of food borne sickness which often leads to diarrhea or hemorrhagic colitis in humans and cause a main of hemolytic uremic syndrome those leaders to acute renal failure in kids (Al-Saadi *et al.*, 2018), and serious problems that cause a great loss to the bird's enterprises all over the world.

E. coli serotype O157: H7 has been isolated of fowl, turkeys and pigeons can transference shigatoxin-creating in *E. coli* (STEC), and affect to public health (Nolan *et al*., 2015). Extra epidemiological researches are necessary in order to control the potential role of chicken and cow as reservoir of *E. coli* O157:H7 (Osman *et al*., 2017). *E. coli* isolates showed that resistance developed to some antibacterial with passage of time progress of resistance may be because of the vigorous use of these antibiotics for control of diseases in poultry and also due to use of antibacterial as feed additives (Profile, 2017). *E. coli* is a chief global society health concern for the reason that it is becoming resistant to at present available antibiotics (Subashchandrabose *et al*., 2014).

Materials and Methods

Collection of samples

A total 250 samples collected from poultry fields in the holy Karbala that divided into: 50 stool sample from human and 200 fecal swabs from the chicken cloacae which involve to 100 swabs from broiler chickens and 100 swabs from Laying chicken. All samples were collected by using sterile swabs, placed in sterile tubes contain (10) ml of Buffer Peptone Water and placed in cool box for transporting to the laboratory.

Isolation and Identification of *E. coli*

Enrichment all Samples later collection were placed inside (10) ml of Buffer Peptone Water (BPW) (Oxoid, England) and incubated at 37°C pro overnight followed then subculture.

Culture media preparation: All media were prepared and sterilized according to the company.

Eosin methylene blue agar (EMB) : It was preparation and sterilized according for the company(Lab M limited, UK), poured into petri dishes inside a sterilized hood and preserved into a refrigerator on 4°C, (EMB) used to identification of *E. coli*.

Sorbitol MacConkey Agar : Selective and differential media in support of the isolation of O157:H7 after preparation according to the company manufacturer, One Loop full bacteria taken from a 24h fresh (EMB) culture and cultured on SMAC(TM MEDIAD, India) after that incubated

at 37°C pro 18 hr, *E. coli* O157:H7 were appears colorless colony (nonsorbitol fermenter).

Chrom agar O157: Use the chromogenic media for isolation and diagnosis of *E. coli* serotype O157:H7(Fadhil & Yousif., 2018). Bacteria appear, as different colonies in different colors, preparation the media according to the company manufacturer (HiChrom™ EC O157H7, India).

Biochemical tests: The identefiction the suspected *E. coli* O157:H7 was made via biochemical tests involving conservative glucose and lactose fermentation via Triple suger iron test (TSI) ,urease, and IMViC test involved (indol, methyl red, Voges proskauer, citrate utilization) (Quinn *et al.*, 2011).

PCR detection method:

Method of DNA extraction and purification: the Genomic DNA was isolated from bacterial grow according to the protocol of ABIO pure Extraction, kit (Promega, USA).The conventional PCR was performed to identify *E. coli* O157:H7 on 16srRNA, rfbO157 and fliCh7 genes were investigated by (Alpha DNA Company, Canada), a fragment size of genes, 1500 bp, 420 bp and 625 bp of (16srRNA, rfb O157 and fliCh7) genes respectively.

Reaction Setup and Thermal Cycling Protocol (A): by a total volume of 25µl, which involved, 12.5 µl of PCR, 1 µl of every (forward and reverse) primer, Nuclease Free Water 7.5 µl and 3 µl of template DNA Table (2).

After PCR amplification, (1%) the gel agarose electrophoresis used was adopted to confirm the existence of amplification. PCR products were sent pro Sanger sequencing using ABI3730XL, automated DNA sequences, through Macrogen Corporation – Korea.

Reaction Setup and Thermal Cycling Protocol (B): by a total volume of 20µl, which involved, 10 µl of PCR, 1 µl of every forward and reverse primers, Nuclease Free Water 4 µl and 4 µl of template of DNA Table (3).

Table 1 : PCR primer with their Nucleotide sequences and amplicon size

	Target gene	Primers Sequence	Product Size (bp)	Reference
A	16srRNA	F /AGAGTTTGATCCTGGCTCAG R / TACGGTTACCTTGTTACGACTT`	1506	(Lane, 1991)
B	rfbO157	F / GGTGATGATGTTGAGTTG R / AGATTGGTTGGCATTACTG	420	(Maurer *et al*., 1999)
	fliCh7	F/ GCGCTGTCGAGTTCTATCGAGC R/CAACGGTGACTTTATCGCCATTCC	625	(Alonso *et al*., 2007)

Table 2 : The optimum condition of detection 16srRNA gene

Steps	°C	Time	No. of cycle
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing	60°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	7 min	1
Hold	4°C	10 min	

Table 3 : The optimum condition of detection rfbO157and fliCh7 genes

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	63	00:30	
Extension	72	00:60	
Final extension	72	05:00	1
Hold	10	10:00	

Sequencing and sequence Alignment

After confirming the amplification *E. coli* O157:H7 via conventional PCR, PCR product were sent to Sanger sequencing utilizing ABI3730XL, automated DNA sequences, through Macrogen Corporation – Korea to determine the DNA Sequencing in these genes. Homology search was conducted utilizing Basic Local Alignment Search Tool (BLAST) program, which is obtainable at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov and bio Edit program. Consequences were compared with data got of Gene Bank published EXPASY program which is obtainable at the NCBI online.

Antimicrobial susceptibility

The antimicrobial sensitivity testing *E. coli* serotype O157: H7 isolates were behaved utilizing disc diffusion way on Mueller Hinton agar (Himedia, India) accord to principles of Clinical and Lab. Standards Institute (CLSI) (Weinstein *et al*., 2019), *E. coli* serotype O157: H7 positive isolates were estimated pro antimicrobial sensitivity to 8 antimicrobial agents: Gentamycin (10 µg), amoxicillin (30µg), chloramphenicol (30 µg), ampicillin (10 µg), streptomycin (25 µg), tetracycline (30 µg), Doxycycline (10 µg) and Neomycin (10 µg) (Bioanalyse/ turkey). The McFarland 0.5 (turbidity of the test broth was set together with saline till the cloudiness of the test suspension associated that of the standard) standardized suspension of the bacteria into

tryptone soya broth (Himedia, India) was made. A bacterial suspension incubated pro 6–8 hrs. was wipe above the entire surface of Mueller-Hinton agar (Himedia (India) by a sterile cotton wipe, the inoculated plates were permitted to stand 3–5 minutes to watch any a plus moisture of the medium afore the antimicrobial discs were used , a ring of discs containing sole concentrations of every antimicrobial agent, and put on the inoculated surface utilizing disc distributor, gently compressed with the point of the forceps ensuring total connect with the agar surface, and after that overturned, Clear zones made via antimicrobial inhibition of bacterial grow were measured in mm utilizing a measuring caliper, next 16-18 hrs, of incubation at 37°C aerobically, to the sensitivity testing. To end, the outcomes of antimicrobial resistance testing were noted as resistant, susceptible and intermediate agreeing to CLSI break points (Weinstein *et al*., 2019).

Results

A total of 3(6%) positive sample of *E. coli* serotype O157: H7 were isolated from 50 stool specimen selected from human (workers in chicken fields), Positive sample 1(1%) were isolated of cloacal swab in 100 sample of laying, 5(5%) samples were isolated from 100 sample of broiler show taken from poultry fields in the holy Karbala city, Iraq according to culture methods, biochemical tests and PCR using rfbO157 gene, that amplified from human and chicken isolates via using conventional PCR a product size was approximately 420 bp. and using fliCh7 gene that amplified a product size was approximately 625 bp Fig.(1). Also using 16s RNA gene, which amplified a product size of about 1500bp. Fig. (2). as shown in the table (4).

Table 4 : Percentage of *E. coli* serotype O157:H7 isolate's thru using culture methods, biochemical tests and PCR

Isolation results	+ve No.	%	- ve No.	%	X^2 value
Workers	3	6	47	94	Calculated X^2=3.34
Broiler	5	5	95	95	Tabulated X^2 =5.99
Laying	1	1	99	99	df= 2
Total	9		241		non-significant (P>0.05)

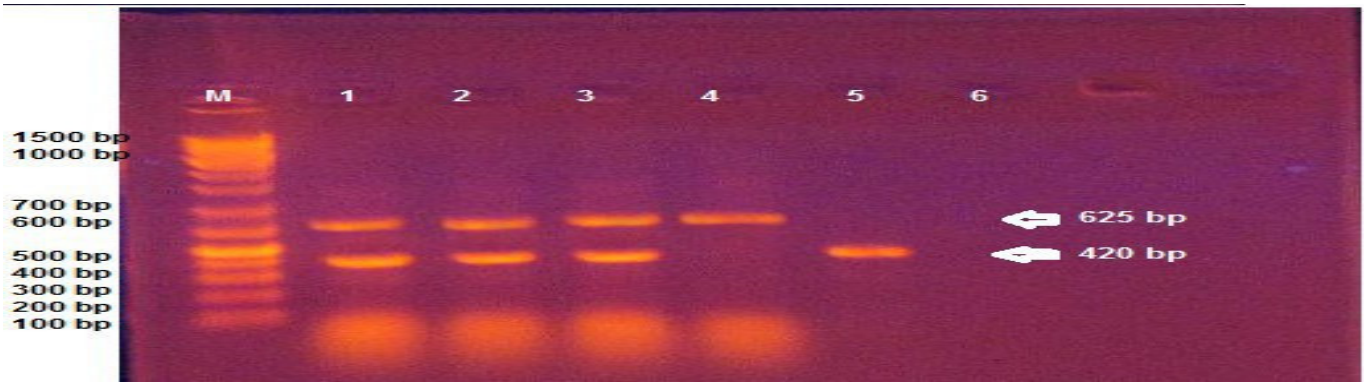

Fig. 1 : Agarose gel electrophoresis shows amplification product of 420 bp and 625 bp fragments of (rfbO157 and fliCh7) genes by PCR. (2%) agarose gel (5 volt\ cm2\ 1.5 hours) Lane M: Marker ladder (100-1500 bp) Lanes: 1 to 3 shows the product PCR of human, Lanes: 4 and 5 show the product PCR of *E. coli* O157:H7 from chicken.

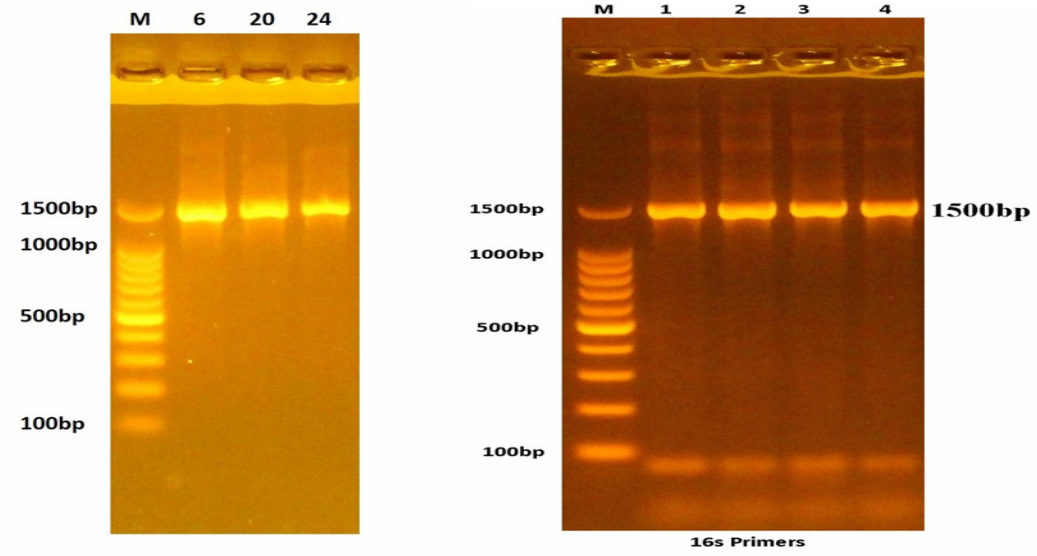

Fig. 2 : Agrose gel electrophoresis for 16s r RNA gene 1500bp. Bands were fractionated by electrophoresis on a 1% agarose gel (1h, 5 volt\cm2 1X TBE) and visualized under U.V light after staining with Eth.Br. M (100 - 1500 bp Marker ladder). Lane 4, 3 :16s r RNA gene in human, Lane 2 :16s r RNA gene in laying hens chicken, Lane 1, 6, 20, 24: 16s r RNA gene in broiler chickens.

Sequence analysis pro *E. coli* serotype O157: H7

A total of 9 isolated the confirm used monoplex PCR technique pro detection genes of *E. coli* serotype O157: H7 in stool samples by use rfbO157 and fliCh7genes Fig (1), 7 of them were confirmed by using small subunit ribosomal PCR gene (16s rRNA gene) amplified by a specific primer Fig (2).

Tow stool samples from humans, one from laying hens and four fecal samples from broiler chickens of *E. coli* O157: H7. Sequencing and analysis for similarities across GenBank, sepsi Ttest and EzTaxaon, some isolates showed a 100% nucleotide sequence identity, and other isolates gave 99% similarity sequences. Sequencing and analysis isolates in a table (5).

Table 5 : Appear isolates of *E. coli* O157:H7 by 16s rRNA gene sequencing ID in gene bank and nucleotide sequence identity from (NCBI).

Local isolates	Accession numbers for local isolates	NCBI-BLAST Homology Sequence Identity (%)		
		Accession numbers	Country	Identity
E. coli O157: H7 No.1	-	AM184233.1	GERMANY	100%
E. coli O157: H7 No.2	-	AM184233.1	GERMANY	100%
E. coli O157: H7 No.3	MT782290.1	AM184233.1	GERMANY	100%
E. coli O157: H7 No.4	MN689684.1	AM184233.1	GERMANY	100%
E. coli O157: H7 No.5	MT370810.1	CP031913.1	Canada	99 %
E. coli O157: H7 No.6	MT370824.1	AM184233.1	GERMANY	100%
E. coli O157: H7 No.7	MT782280.1	AM184233.1	GERMANY	100%

Seven *E. coli* O157 H7 isolates were obtained after DNA sequencing, tow from human origin were submitted to NCBI gen bank at accession number MT370824.1 and MT782290.1, one form layer origin have accession number MT370810.1, and two from broiler origion have accession number MN689684.1 and MT782280.1 while the other two types remaining have similar nucleotides sequences 100% identity.

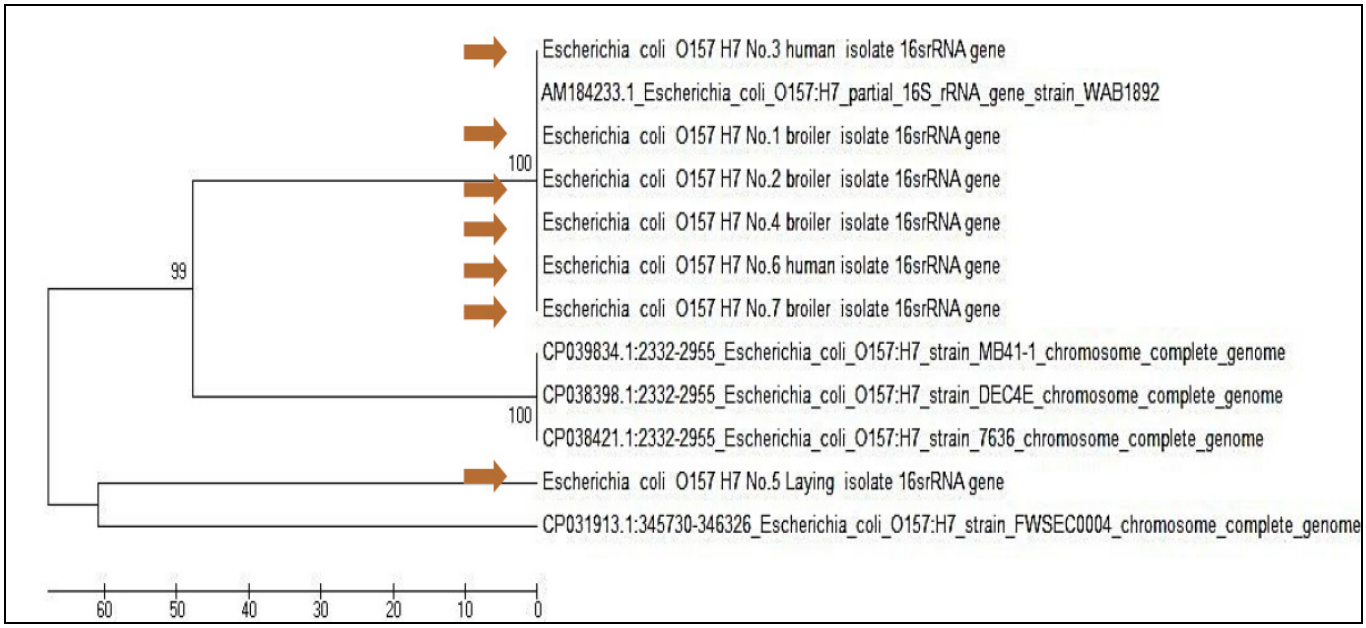

Fig. 3: phylogenetic tree analysis based on partial sequence of16srRNA gene in local *E. coli* O157 H7 isolates that used for genetic comfirmative detection analysis. The evolutionary distances were computed using Maximum Composite Likehood method by UPGMA phylogenetic tree (MEGA 6.0 version).The local *E. coli* O157 H7 isolates were show closed related to NCBI- Blast *E. coli* O157 H7 (AM1842233.1).

Antibiotic susceptibility of human *E. coli* serotype *O157:H7* isolates

The isolates *E. coli* serotype O157:H7 from human showed that all isolated were Susceptible 66.6% to (Gentamicin, Streptomycin and Chloramphenicol) low Susceptible to other antibiotics, in contrast absolute resistant 100% against Ampicillin, Tetracycline and Amoxicillin 66.6%, Gentamicin, Neomycin, Chloramphenicol and Doxycycline 33.3% Intermediate Susceptible were reported in Neomycin, Streptomycin, Doxycycline 33.3 % in table (6). Fig (4) and Fig (5).

Table 6 : Results of antibiotic susceptibility testing of human *E. coli* O157: H7.

Antibiotic	Abbreviation	Concentration (µg/ disc)	Diameter of the inhibition zones			Number of isolation		
			Susceptible	Intermediate	Resistant	Susceptible %	Intermediate %	Resistant %
Gentamicin	CN	10	≥15	13-14	≤12	2(66.6)	0	1(33.3)
Neomycin	N	10	≥15	13–14	≤12	1(33.3)	1(33.3)	1(33.3)
Ampicillin	AM	10	≥17	14–16	≤13	0	0	3(100)
Tetracycline	TE	30	≥15	12–14	≤11	1(33.3)	0	2(66.6)
Streptomycin	S	25	≥15	12–14	≤11	2(66.6)	1(33.3)	0
Amoxicillin	AX	30	≥17	14–16	≤13	1(33.3)	0	2(66.6)
Chloramphenicol	C	30	≥18	13–17	≤12	2(66.6)	0	1(33.3)
Doxycycline	DO	10	≥14	11–13	≤10	1(33.3)	1(33.3)	1(33.3)

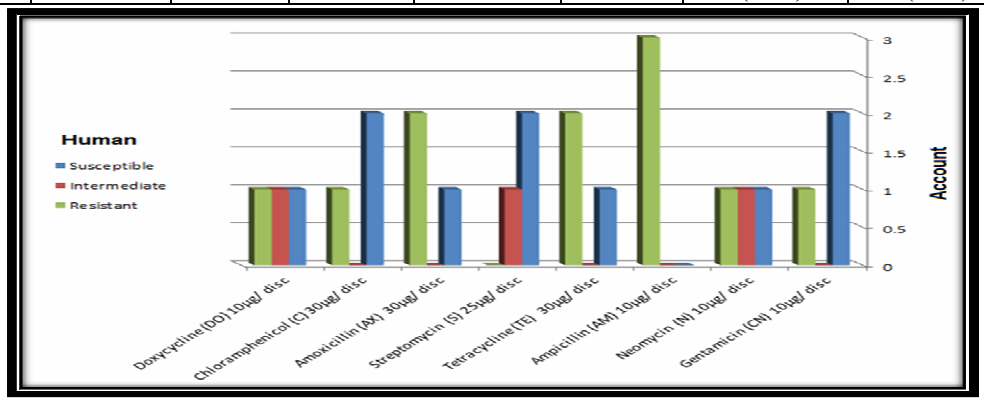

Fig. 4 : Bar diagram showing antibiotic susceptibility testing pattern of human *E.coli* O157:H7 isolates.

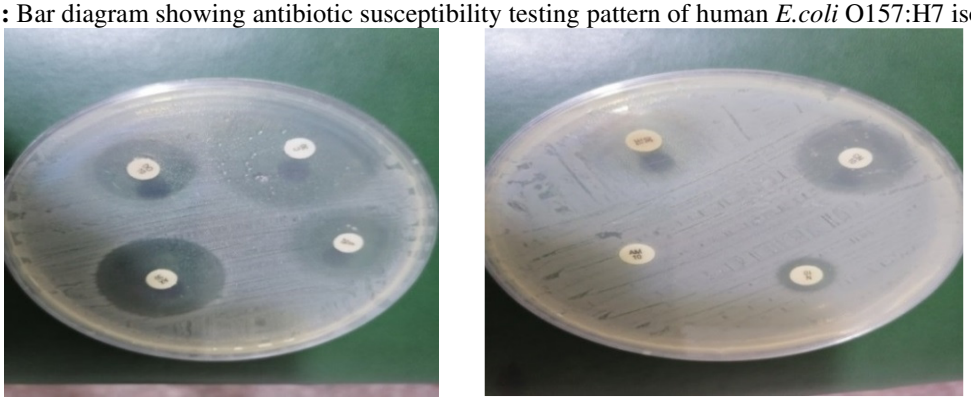

Fig. 5 : Antibiotic susceptibility testing of human *E. coli* O157: H7 isolates Mueller Hinton agar

Antibiotic susceptibility of chicken *E. coli* O157:H7 isolates

The isolates *E. coli* O157:H7 from chicken showed that all isolated were Susceptible 50% to (Gentamicin and Streptomycin) low Susceptible to other antibiotics, in contrast absolute resistant 100% against (Ampicillin and Amoxicillin), Neomycin and Doxycycline 83.3 %, Tetracycline 66.6%, Gentamicin, Chloramphenicol 50%, Streptomycin 33.3%, intermediate Susceptible were reported in Chloramphenicol, Streptomycin, 16.6 % Table (7), Fig (6) and Fig (7).

Table 7 : Results of antibiotic susceptibility testing of chicken *E. coli* O157: H7.

Antibiotic	Abbreviation	Concentration (µg/ disc)	Diameter of the inhibition zones			Number of isolation		
			Susceptible	Intermediate	Resistant	Susceptible%	Intermediate%	Resistant%
Gentamicin	CN	10	≥15	13-14	≤12	3(50)	0	3(50)
Neomycin	N	10	≥15	13–14	≤12	1(16.6)	0	5(83.3)
Ampicillin	AM	10	≥17	14–16	≤13	0	0	6(100)
Tetracycline	TE	30	≥15	12–14	≤11	2(33.3)	0	4(66.6)
Streptomycin	S	25	≥15	12–14	≤11	3(50)	1(16.6)	2(33.3)
Amoxicillin	AX	30	≥17	14–16	≤13	0	0	6(100)
Chloramphenicol	C	30	≥18	13–17	≤12	2(33.3)	1(16.6)	3(50)
Doxycycline	DO	10	≥14	11–13	≤10	1(16.6)	0	5(83.3)

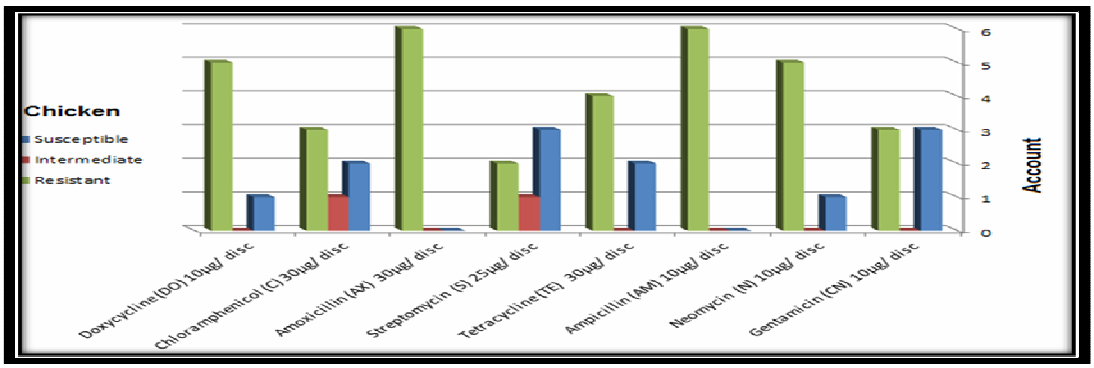

Fig. 6 : Bar diagram showing antibiotic susceptibility testing pattern of chicken *E. coli* O157: H7 isolates.

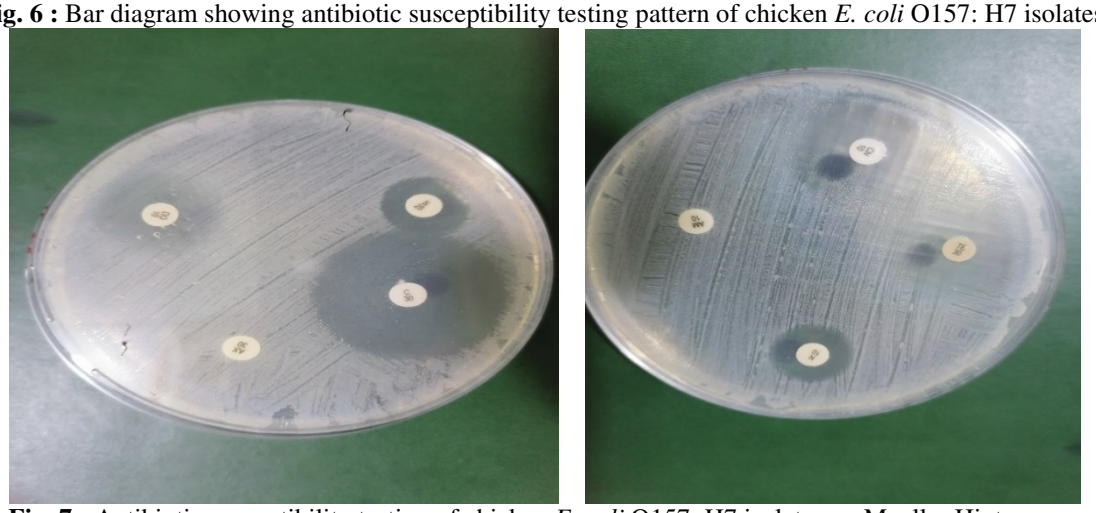

Fig. 7 : Antibiotic susceptibility testing of chicken *E. coli* O157: H7 isolates on Mueller Hinton agar

Discussion

A total of 3(6%) positive sample of *E. coli* serotype O157:H7 were isolated of 50 stool specimen selected from human (workers in chicken fields), as shown in the table (4)These results correspond also other study occurred in holly Karbala governorate collected 230 stool specimen of diarrheic kids showed that 11(4.78%) out of 230 were positive for *E. coli* O157: H7 (Al–Dawmy and Yousif, 2013), and with Ademokoya *et al.* (2013) appeared *E. coli* serotype O157:H7 78 (4.3%) positive from Out of 1800 diarrheic patients sampled. On the other hand, it did non-correspond with the prevalence of (STEC) O157:H: 7 in stool samples was 16 /80 (20%) in holly Karbala governorate (Ali and Jameel, 2018), on the other hand, in Iran not found of the isolated *E. coli* was O157: H7 serotype from a total of 137 stool samples of patients (Khosravi *et al.*, 2016). The prevalence *E. coli* O157: H7 might be due to variation in sample size, geographical variations, seasonal variation, the methodology used for identification, and hygienic precautions

Positive sample 1(1%) were isolated from cloaca swab in 100 sample of laying, 5(5%) samples were isolated from 100 sample of broiler show taken from poultry fields in the holy Karbala city, Iraq. As shown in the table (-), all results in chicken agree with another study conducted in Iran reported that 31 (7.3%) of 422 chick samples were polluted with *E. coli* O157:H7 (Momtaz and Jamshidi, 2013), in contrast researched in Diyala governorate, (Iraq) shown the rate of the numeral of the presence of *E. coli* O157:H7 in broilers carcasses was 50% (Yaseen *et al.*, 2017). This difference may be due to the method of sampling, time, lack of strict sanitary measures between fields and mutual pollution.

Molecular and Sequence analysis of *E. coli* O157:H7 isolated:

In the current study finding all results 9 positive isolated by the PCR demonstrated that PCR exam found *E. coli* O157: H7 by use virulence genes (rfbO157, flicH7 genes). In Amarah city Khanjar and Alwan, (2014) found that all 10 positive *E. coli* O157: H7 expressed by (rfbO157, flicH7 genes), Define their supposed virulence genes by PCR analysis , showed a low number of such isolates. to ensure the validity of the results, forward primers were sent to Macrogen Company in South Korea for DNA sequences. The obtained sequences of these samples were aligened using NCBI software. Furthermore, the nucleotide sequences were compared to the inform. in the gene bank of the NCBI web site databases using the Basic Local Alignment Search Tool (BLAST) and examined by BioEdit software. Numerous studies has used analysis sequencing for identified *E. coli* serotype O157:H7 such as (Alalade *et al.*, 2018)

Our results show when 16srRNA is performed through the sequence of analysis and the genetic tree, the nucleotides of some human isolates with broiler chicken isolates show closed related, while the laying hens appear separate or away from the tree in figure (4-18).This difference may be due to the environmental condition, the difference in the use of antibiotics, or the result of the period of time that the laying hens live more than meat, and this may cause mutations over time.

Antibacterial Susceptibility Test

In human

A total of the 3 positive isolates obtained in this study were resistant to at least 7 of the antibiotic used, with highest resistance rates observed 100% against Ampicillin,

Tetracycline and Amoxicillin 66.6%, Gentamicin, Neomycin, Chloramphenicol and Doxycycline 33.3%, Susceptible 66.6% to (Gentamicin, Streptomycin and Chloramphenicol), the obtained results in this study were corresponding with the consequences of (Moses *et al*., 2012), Islam *et al*. (2016) were reported susceptibility of the *E. coli* isolates was against Gentamicin (92.59%).

Another study non corresponding with the results of a study prepared Adwan *et al*., (2002) found 55% of isolated strains resistant to gentamicin, Other reports showed antibiotic susceptibility verotocytoxin producing *E. coli* O157 were 100% resistant to and chloramphenicol, streptomycin (Raji *et al*., 2008).

In chicken

A total of the 6 positive isolates obtained in this study were resistant to at least 8 of the antibiotic used, with highest resistant 100% against (Ampicillin and Amoxicillin, Neomycin and Doxycycline 83.3%, Tetracycline 66.6%, Gentamicin, Chloramphenicol 50%, Streptomycin 33.3%), the results in this study were a agreement with the results İnanc and Mustafa., (2018), and Altaee (2012), High level of *E. coli* isolates resistant to amoxicillin and ampicillin

So on the results were disagreement with Akond *et al.*, (2009) the present finding low susceptible *E. coli* isolates were reported to streptomycin (30%) , Researchers had reported susceptible serotype O157:H7 isolates to chloramphenicol (100%) Hailu & Tefera, (2016) and Zakeri and Kashefi, (2012), who recorded highly resistant *E. coli* isolates to streptomycin (67%). This may be because of indiscriminate utilize of antimicrobials in veterinary, and agriculture that elevate the emergence and distribution of antimicrobial resistant microorganisms or the utilize of these antimicrobial drugs in diverse regions. the results of study reveals that the intestinal track of chicken harbors the bacterial pathogen hence interventions are needed to reduce transmission of *E. coli* O157:H7, so must be there procedures Public health education and application of strict hygienic measures in chicken fields during deal with chicken, clean the field or collect eggs, so essential to minimalize the risk of cross contamination and spread of infection to workers of chick fields, the risk of human exposure to serotype O157:H7. Furthermore, antibiotic usage should be controlled evading increased antibiotic resistance.

References

Abdela, W. (2017). Characterization of Diarrheagenic *Escherichia Coli* Serotypes Isolated from Poultry and Humans. SOJ Journal Veterinary Sciences, 3 (1): 1–8.

Ademokoya, A.A.; Adebolu, T.T.; Ogundare, A.O.; State, O. and State, O. (2013). Original Research Article Infection rate of *E. coli* O157: H7 among diarrhoeic patients in Southwest Nigeria. 2(10): 524–533.

Adwan, K.; Abu-Hasan, N.; Essawi, T. and Bdir, M. (2002). Isolation and characterisation of Shiga toxigenic *Escherichia coli* strains from northern Palestine. Journal of Medical Microbiology, 51(4): 332–335.

Akond, M.A.; Alam, S.; Hassan, S.M.R and Shirin, M. (2009). Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Internet Journal of Food Safety, 11, 19–23.

Alalade, O.M.; Ameh, J.B.; Abdullahi, I.O. and Whong, C.M.Z. (2018). Screening for virulence genes in *Escherichia coli* O157:H7 obtained from drinking water from Ikara, Kaduna state, Nigeria. *Ife Journal of Science*, *20*(1): 139.

Ali, H. and Jameel, Y.J. (2018). Comparative Study between conventional and molecular assays for detection Shiga toxin-producing Escherichia Coli in children with bloody diarrhea in Kerbala city . International Journal of Advanced Biological Research , 8(1): 89-93.

Alonso, S.; Mora, A.; Blanco, M.; Blanco, J.E.; Dahbi, G.; Ferreiro, M.T.; López, C.; Alberghini, L.; Albonetti, S. and Echeita, A. (2007). Fecal carriage of *Escherichia coli* O157: H7 and carcass contamination in cattle at slaughter in northern Italy. International Microbiology, 10(2): 109

Altaee, A.E. (2012). Detection of *E. coli* Serotyping O157:H7 Isolated from Beef and Poultry Products Using Polymerase Chain Reaction. Master Thesis in Vet. Public Health, Collage of Vet.; Medicine. Mosul University, Pp. 56.

Al-Dawmy, F.A.A. and Yousif, A.A. (2013). Prevalence of *E.coli* O157:H7 in intestinal and Urinary tract infection in children. International Journal of Advanced Research , 1(8): 111-120.

Al-Saadi, Z.H.; Tarish, A.H. and Saeed, E.A. (2018). Phenotypic detection and antibiotics resistance pattern of local serotype of *E. coli* O157:H7 from children with acute diarrhea in hilla city/Iraq. Journal of Pharmaceutical Sciences and Research, 10(3): 604–609.

Ali Hussein, F. and Abdulrahman Yousif, A. (2018). *Immunization of lambs vaccinated with Escherichia coli O157:H7*.

Hailu, D. and Tefera, G. (2016). Isolation and characterization of multidrug resistant *Escherichia coli* isolates from contagion syndrome poultry farm. International Journal of Current Trends in Pharmacobiology and Medical Sciences, 1(2): 19–26.

İnanç, A. and Mustafa, A.S. (2018). Antibiotic Resistance of *Escherichia coli* O157: H7 Isolated from Chicken Meats. Tarim ve Doga Dergisi, 21(1): 7.

Islam, M.M.; Ahamed, S.; Arafat, M.Y.; Hasan, I.; Rahman, M. and Nazir, K. (2016). Molecular detection and antibiogram of shiga toxin producing *Escherichia coli* (STEC) isolated from diarrheic children. *Bangladesh Journal of Veterinary Medicine*, 14(2): 289–295.

Khanjar, A.F. and Alwan, M.J. (2014). Genotypic study of Escherichia coli O157: H7 isolated from stool samples of humans and cattle. Int. J. Adv. Res, 2(6): 204-212.

Khosravi, A.D.; Khaghani, S.; Sheikh, A.F.; Ahmadzadeh, A. and Shamsizadeh, A. (2016). Prevalence of *Escherichia coli* O157: H7 in children with bloody diarrhea referring to abuzar teaching hospital, Ahvaz, Iran. *Journal of Clinical and Diagnostic Research,* 10(1): DC13-DC15.

Lane, D.J. (1991). 16S/23S rRNA sequencing. *Nucleic Acid Techniques in Bacterial Systematics*, 115–175.

Lim, J.Y.; Yoon, J.W. and Hovde, C.J. (2010). A brief overview of *Escherichia coli* O157: H7 and its plasmid O157. Journal of Microbiology and Biotechnology, 20(1): 5.

Maurer, J.J.; Schmidt, D.; Petrosko, P.; Sanchez, S.; Bolton, L. and Lee, M.D. (1999). Development of primers to O-antigen biosynthesis genes for specific detection of *Escherichia coli* O157 by PCR. Appl. Environ. Microbiol. Messens, 65: 2954-2960.

Momtaz, H. and Jamshidi, A. (2013). Shiga toxin-producing *Escherichia coli* isolated from chicken meat in Iran: Serogroups, virulence factors and antimicrobial resistance properties. Poultry Sci.; 92: 1305-1313.

Moses, A.E.; Egwu, G.O. and Ameh, J.A. (2012). Antimicrobial resistant pattern of *E. coli* O157 isolated from human, cattle and surface water samples in northeast Nigeria. J. Vet. Adv, 2(5): 209-215.

Nolan, L.K.; Barnes, H.J.; Vaillancourt, J.P.; Abdul-Aziz, T. and Logue, C.M. (2015). Colibacillosis . In: Manual of Poultry Diseases, edited by Brugere-Picoux, J.; Vaillancourt, J. P.; Shivaprasad, H. L.; Venne,D.; and Bouzouais, M. AFAS, 301-315.

Osman, A.l.; Işıl, A.R.; Melda, M.; Başak, B. and Behzad, H.F.K. (2017). Prevalence of *Escherichia coli* isolated from meat, chicken and vegetable samples in Turkey Journal of Biotechnology Science Research. 2017; 4(3): 214-222.

Profile, S.E.E. (2017). Isolation of Escherichia coli from Poultry Liver and its Antibiogram Profile. *March*. https://doi.org/10.17582/journal.rjvp/2017/5.1.12.14

Quinn, P.J.; Markey, B.K.; Leonard, F.C.; Hartigan, P.; Fanning, S. and Fitzpatrick, Es. (2011). *Veterinary microbiology and microbial disease*. John Wiley & Sons.

Raji, M.A.; Minga, U.M. and Machang'u, R.S. (2008). Prevalence and characterization of verotocytoxin producing *Escherichia coli* O157 from diarrhoea patients in Morogoro, Tanzania. Tanzania Journal of Health Research, 10(3): 151-158.

Subashchandrabose, S.; Hazen, T.H.; Brumbaugh, A.R.; Himpsl, S.D.; Smith, S.N.; Ernst, R.D.; Rasko, D.A. and Mobley, H.L.T. (2014). Host-specific induction of Escherichia coli fitness genes during human urinary tract infection. Proceedings of the National Academy of Sciences, 111(51): 18327–18332.

Weinstein, M.P.; Patel, J.B.; Bobenchik, A.M.; Campeau, S.; Cullen, S.K. and Galas, M. (2019). Performance standards for antimicrobial susceptibility testing performance standards for antimicrobial susceptibility testing. CLSI, Suppl M, 100, 1-25.

W.H.O. (World Health Organization) (2016): *E. coli* O157 Fact Sheets Updated October 2016.

Yaseen, S.M.; Saleh, A.M. and Al-Zubaidy, R.S.A. (2017). Contamination of the local produced broilers carcasses with *Escherichia coli* O157: H7 and its effect in public health in Diyala province. The Iraqi Journal of Veterinary Medicine, 41(2): 113-117.

Zakeri, A. and Kashefi, P. (2012). Isolation and drug resistance patterns of *Escherichia coli* from cases of colibacillosis in Tabriz. Journal of Animal and Veterinary Advances, 11(19): 3550–3556