International Journal of Pharmaceutical and Medical Research
Volume – 3 Issue – 2 April 2015
Website: www.woarjournals.org/IJPMR *ISSN: 2348-0262*

Isolation, Antibiotic resistance Patterns and Plasmid Profile of *E. coli* 0157:H7 from Environmental Samples in Basrah Governorate– Iraq

Maytham A. Al-Hamdani[1], Abbas Dareb shaban[2]

[1]Department of Biology – College of Education For Pure Sciences – University of Basrah, Iraq.

[2]Department of Biology – College of Education For Pure Sciences – University of Basrah, Iraq.

Abstract: **Escherichia** ***coli O157:H7 is considered as a public health concern. The present study attempt to isolation of this serotype from environment samples (Swage waste water , river water and tap water from different sites in Basrah governorate Iraq between August 2013 – October 2014. Out of 281 samples, 40 (14.23 %) of samples were found positive for this bacterium when plted on chromogen agar and (CT-SMA). The isolates then identified using conventional tests confirmed using certestE.coli O157:H7. Antibiotic susceptibility test were performed by Bauer-Kirby disc diffusion assay against (20) antibiotics . the present study revealed that all the isolates showed high resistance to Ampicillin, Augmentin , Piperacillin , Cefazolin and rifampin. Variable degree of susceptibility against other antibiotics.***

The present data showed different antibiotic resistance patterns suggesting that these patterns are isolate dependent.Plasmid profile of the isolates revealed that the more resistant isolate to different antibiotics the more number of plasmids harbored by the isolate.

Keywords: *E. coli* 0157:H7,Antibiotic resistance Patterns ,Plasmid Profile.

1. Introduction

It is well – known that *Escherichia coli* is usually normal commensal bacterium in the intestinal tract of humans and animals. Pathogenic serotypes cause intestinal and extraintestinal infections. One of the intestinal infections that cause diarrhea or hemorrhagic colitis in human attributed to enterohemorrhagic *E.coli* that may progress to hemolytic uremic syndrome in children and adults [1]. *Escherichia coli* 0157:H7 serotype is the cause in most of these cases due to the mostly acquiring the capacity to produce St_{x2a} toxin [2]. Transmission of infection is well – known by this bacterium is by fecal – oral route through contaminated food, water or by direct contact with reservoir hosts particularly cattle.

Despite *E. coli* 0157:H7 considered as public health concern in most countries of the world, few studies are available on this bacterium in Iraq. Most of these studies restricted on samples taken from human, attempt to isolate, and characterize the bacterium from human and animals or from milk [3]. No data available concerned about the isolation, antibiotic susceptibility patterns and plasmid profile of *E. coli* 0157:H7 from environmental samples.

The present study was conducted to isolate *E. coli* 0157:H7 from environmental samples (Sewage, wastewater, tap water, river waters collected from different sites in Basrah Province – Iraq and studying plasmid profile and antibiotic susceptibility to antibiotics.

2. Materials & Methods

2.1 Samples Collection:

Tap water, sewage wastewater and river water samples were collectedfrom different governorate sites inBasrah during the periodfromAugust 2013 toOctober 2014in disposable plastic bottles and brought to the laboratory directly.

2.2. Isolation of E. coli 0157:H7 on selective media:

Water sample (0.1 ml) was spread onto the surface of selective media chromogenic agar plates and on Cefixime–tellurite sorbitol MacConkey Agar (CT-SMA) plates and incubated for 24 hours at 37 C.

Colonies with pink – colour that appeared on Chromogenic Agar plates were picked and further confirmation using Dipstick(certest*E.coli* O157:H7), and kept on Nutrient Agar plates for further studies.

2.3. Antibiotic Sensitivity Test:

Antibiotic susceptibility profiles of *E. coli* isolates were determined by the standard Kirby–Bauer disk diffusion method [4].The antibiotics with their respective disk con-centrations are as follows: Ampicillin (10 µg), Amoxacillin + Augmentin (30µg), Cefazolin (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), imipenem (10 µg), norfloxacin (10 µg), piperacillin (100 µg), Ceftriaxone (30µg), and Aztreonam (30µg), Amikacin (10 µg), Kanamycin (30 µg), Ciprofloxacin (5 µg), rifampin (300µg), Tetracycline (25 µg), Cefoxitin (30µg), Tobramycin (10µg), Cefepime (30 µg) , Cefixime (30 µg) , doxycycline (30µg) . Bacterial cultures suspension(0.1 ml)equivalent to 0.5 McFarland turbidity standards were spread on Mueller-Hinton agar plates using sterile swabs and incubated aerobically at 37°C for 24 hours, and then the diameter of inhibition zones around antibiotic disks were measured. Results were expressed susceptible or resistant according to the criteria recommended by the [5].

2.4. Isolation of Plasmid DNA and Agarose Gel Electrophoresis:

The method of [6] was employed for plasmid screening. The DNA were electrophoresed on 0.8% agarose gel, stained with ethidium bromide, visualized by UV transillumination and photographed. Molecular weights were calculated based on molecular weight standard.

3. Results:

3.1. Environmental Samples:

Two hundred and eighty one environmental samples (121 drinking water samples, 80 samples of sewage waste water, and 80 river water samples). Fourteen samples (14.23%) out of 281 gave positive culture for *E. coli* 0157:H7 by selective cultural media.

Nine samples (7.4%) out of 121 drinking water samples gave positive results for *E. coli* 0157: H7, eighteen (22.5%) of sewage water samples out of 80 samples also gave positive results for *E. coli* 0157: H7. While, thirteen (16.25%) samples carry the organism out of eighty river water samples (Table 1).

Table(1) The numbers and percentages of positive Samples of *E. coli* O157: H7 isolated from different environmentsources in Basrah.

	Negative Samples	positive Samples	Total
SewageWaste water	62(77.5)	18(22.5)	80(100)
River water	67(83.75)	13(16.25)	80(100)
Drinking water	112(92.6)	9(7.4)	121(100)
Total	241(85.77)	40(14.23)	*281(100)*

3.2. Antibiotic Susceptibility of environmental *E. coli* 0157: H7:

Susceptibility of *E. coli* 0157: H7 isolates from the environments to twenty antibiotics.

3.3. Sewage Waste Water

The data obtained revealed that *E. coli* 0157: H7 isolated from sewage waste water from different sites in Basrah governorate, are highly resistant to the antibiotics tested, 100% for Ampicillin and Augmentin, Cephazolin, Rifampin and Piperacillin and resistant to Tetracycline, Cefoxitine, Cefixime and Gentamicin (72.22%, 66.66%,61.11%,44.44%)

All the isolates were susceptible 100% for the antibiotics Kanamycin, Amikacin variable susceptible to chloramphenicol 94.44% Noxfloxacin, Ciprofloxacin, Aztreonam (88.88%), Tetracycline (83.33), Imipenen(72.22%), Cefepime (50%) (Table 2).

3.4. River water:

E. coli 0157: H7 isolated from river water samples were found to be highly resistant to Ampicillin, Augmentin, Piperacillin, Rifampin and Cefazolin. (100% ,100%, 92.30%, 92.30 ,92.30)The isolates were susceptible to the following antibiotics Norfloxacin, Ciprofloxacin, Aztreonam and Amikacin (100% ,100% ,100%,100%), and to less extent Kanamycin. Variable results showed by bacterial isolates to the remaining antibiotics,(Table 3).

3.5. Tap water:

E. coli 0157: H7 isolates from tap water samples were also found to be highly resistant to Ampicillin, Augmentin, Cefazolin, Rifampin Piperacillin(100 %,100% ,100% ,100% ,88.87%)and Gentamycin. Cefepime(77.78% ,66.67%) these isolates were susceptible to chloramphenicol, norfloxacin, Tobramycin (88.89% ,88.89% ,88.89%). Most of the isolates tend to be mostly susceptible (Table 4).

Table (2) Antibiotic Susceptibility testing of *E. coli* O157: H7 isolated from sewage waste water in Basrah

Antibiotic	Symbol	S (%)	I(%)	R (%)
Ampicillin	AM	(0) 0	(0) 0	**(100) 18**
Augmentin	AMC	(0) 0	(0) 0	**(100) 18**
Cefazolin	CZ	(0) 0	(0) 0	**(100) 18**
Piperacilin	PRL	(0) 0	(0) 0	**(100) 18**
Rifampin	RA	(0) 0	(0) 0	**(100) 18**
Tetracycline	TE	(27.77)5	(0) 0	**(72.22)13**
Cefoxitin	FOX	(22.22) 4	(11.11)2	**(66.66) 12**
Cefixime	CEF	(27.77)5	(11.11)2	**(61.11) 11**
Gentamicin	CN	(33.33)6	(22.22) 4	**(44.44)8**
Doxycyclin	DO	(44.44)8	(16.66)3	**(38.88) 7**
Ceftriaxone	CRO	(38.88)7	(33.33)6	**(27.77)5**
Cefepime	FEP	(50) 9	(22.22) 4	**(27.77)5**
Amikacin	AK	(100) 18	(0) 0	**(0) 0**
Kanamycin	K	(100) 18	(0) 0	**(0) 0**
Chloramphenicol	C	(94.44) 17	(5.55)1	**(0) 0**
Ciprofloxacin	CIP	(88.89)16	(5.55)1	**(5.55)1**
Norfloxacin	NOR	(88.89) 16	(5.55)1	**(5.55)1**
Aztreonam	ATM	(88.89) 16	(5.55)1	**(5.55)1**
Tobramycin	TOB	(83.33) 15	(5.55)1	**(11.11)2**
Imipenen	IPM	(72.22) 13	(11.11)2	**(16.66)3**

Table (3) Antibiotic Susceptibility testing of*E. coli* O157: H7 isolated from River water in Basrah

Antibiotic	Symbol	S (%)	I(%)	R (%)
Ampicillin	AM	(0) 0	(7.69) 1	**(92.30) 12**
Augmentin	AMC	(0) 0	(7.69) 1	**(92.30) 12**
Cefazolin	CZ	(0) 0	(0) 0	**(100) 13**
Piperacilin	PRL	(0) 0	(7.69) 1	**(92.30) 12**
Rifampin	RA	(0) 0	(0) 0	**(100) 13**
Tetracycline	TE	(61.53)8	(15.38) 2	**(23.07) 3**
Cefoxitin	FOX	(7.69) 1	(0) 0	**(92.30) 12**
Cefixime	CEF	(23.07)3	(53.84)7	**(23.07)3**
Gentamicin	CN	(15.38)2	(7.69) 1	**(76.92)10**
Doxycyclin	DO	(61.53)8	(23.07)3	**(15.38)2**
Ceftriaxone	CRO	(30.76)4	(27.77)5	**(30.76)4**
Cefepime	FEP	(30.76)4	(61.53) 8	**(7.69)1**
Amikacin	AK	(100) 13	(0) 0	**(0) 0**
Kanamycin	K	(61.53)8	5 (27.77)	**(0) 0**
Chloramphenicol	C	(69.23) 9	(7.69)1	**(23.07) 3**
Ciprofloxacin	CIP	(100) 13	(0) 0	**(0) 0**
Norfloxacin	NOR	(100) 13	(0) 0	**(0) 0**
Aztreonam	ATM	(100) 13	(0) 0	**(0) 0**
Tobramycin	TOB	(7.69) 1	(15.38)2	**(76.92)10**
Imipenen	IPM	(53.84) 7	(23.07)3	**(23.07)3**

Table (4) Antibiotic Susceptibility testing of E. coli O157: H7 isolated from Tap water in Basrah

Antibiotic	Symbol	S (%)	I(%)	R (%)
Ampicillin	AM	(0) 0	(0) 0	**(100) 9**
Augmentin	AMC	(0) 0	(0) 0	**(100) 9**
Cefazolin	CZ	(0) 0	(0) 0	**(100) 9**
Rifampin	RA	(0) 0	(0) 0	**(100) 9**
Piperacilin	PRL	(0) 0	(11.11) 1	**(88.87) 8**
Gentamicin	CN	(0) 0	(22.22) 2	**(77.78)7**
Cefoxitin	FOX	(33.33) 3	(0) 0	**(66.67) 6**
Cefixime	CEF	(33.33) 3	(0) 0	**(66.67) 6**
Ceftriaxone	CRO	(33.33) 3	(22.22) 2	**(44.44)4**
Imipenen	IPM	(44.44)4	(11.11) 1	**(44.44)4**
Amikacin	AK	(100) 9	(0) 0	**(0) 0**
Chloramphenicol	C	(88.89) 8	(0) 0	**(11.11) 1**
Norfloxacin	NOR	(88.89) 8	(0) 0	**(11.11) 1**
Tobramycin	TOB	(88.89) 8	(11.11) 1	**(0) 0**
Kanamycin	K	(77.78) 7	(11.11) 1	**(11.11) 1**
Ciprofloxacin	CIP	(77.78) 7	(11.11) 1	**(11.11) 1**
Aztreonam	ATM	(77.78) 7	(11.11) 1	**(11.11) 1**
Tetracycline	TE	(55.55) 5	(22.22) 2	**(22.22) 2**
Doxycyclin	DO	(33.33) 3	(55.55) 5	**(11.11) 1**
Cefepime	FEP	(0) 0	(66.67) 6	**(33.33) 3**

3.6. Antibiotic resistance Patterns of Environmental *E. coli* 0157:H7

It is clear from the present results that *E. coli* 0157: H7 environmental isolates can be considered a multidrug resistant pathogen. Theisolates showed resistantto 6 or more up to fifteen antibiotics.

3.7. Antibiotic Resistant Patterns of Environmental*E. coli*O157:H7

The isolates of the bacterium from sewage waste water in the present study showed eighteen antibiotic resistant patterns (Table 5), while that from river water showed twelve pattern and nine antibiotic patterns obtained from *E. coli* 0157: H7 isolates from drinking water.(Table 6)and(Table 7).

3.8. MAR Index of Environmental *E. coli* 0157:H7

Among the different environmental *E. coli* 0157: H7 isolates MAR index was the highest in the isolates from sewage wastewater (0.75) one isolate resistant to 15 antibiotics. MAR index was lower for the isolates obtained from tapwater (0.55) andriver water (0.5)

3.9. Plasmid Profile of Environmental *E. coli* 0157:H7 Isolates:

The present study revealed that all *E. coli* O157:H7 isolates from the environment harboured one or more plasmids of different molecular weights . Sewage waste water isolatesharboured one or more plasmids . It is evident from the present results that there is a correlation between the number of plasmids and the number of antibiotics resist . One isolate showed resistance to 15 antibiotic found to harbourfourplasmids.

Most of the isolates(5 isolates) harboured at least one plasmid of molecular weight more than 10 kb.(Go To Fig .1,2 and 3).

Table(5) Antimicrobial resistance Patterns of 18 Isolates of *E. coli* O157:H7 from sewage waste water in Basrah

Number of antibiotics	Number of isolation	Resistance Pattern Resistance Pattern
5	1	AM,AMC,CZ,RA,FOX
6	1	AM,TE,CZ,RA,DO, CN
6	1	AM,AMC,CZ,RA,PRL,CFM
7	1	AM,AMC,CZ,RA,PRL,TE,CN
7	1	AM,AMC,CZ,RA,PRL,CRO,FOX
7	1	AM,AMC,CZ,RA,PRL,FOX,CN
8	1	AM,AMC,CZ,RA,PRL,TE,CN ,CFM
8	1	AM,AMC,CZ,RA,PRL,TE,CN,FOX
8	1	AM,AMC,CZ,RA,PRL,TE,FEP,DO
8	1	AM,AMC,CZ,RA,PRL,TE,FOX ,CFM
9	1	AM,AMC,CZ,RA,PRL,CFM,DO,CN,FOX
9	1	AM,AMC,CZ,RA,PRL,TE,FOX ,CFM,IPM
10	1	AM,AMC,CZ,RA,PRL,TE,CFM,DO,CN,FOX
10	1	AM,AMC,CZ,RA,PRL,TE,CFM,DO,CRO,FOX
10	1	AM,AMC,CZ,RA,PRL,TE,CFM,DO,TOP,FOX
10	1	AM,AMC,CZ,RA,PRL,FEP,CFM,ATM,CRO,FOX
13	1	AM,AMC,CZ,RA,PRL,TE,FEP,CFM,DO,CRO,FOX,CN,IPM
15	1	AM,AMC,CZ,RA,PRL,TE,FEP,CFM,DO,CRO,FOX,CN,TOP,CIP,NOR

(Table 6). Antimicrobial resistance Patterns of 13 Isolates of *E. coli* O157:H7 from River water in Basrah in Basrah

Number of antibiotics	Number of isolation	Resistance Pattern
7	2	AM,AMC,CZ,RA,PRL,FOX,CN
7	1	AM,AMC,CZ,RA,PRL,IPM,CN
7	1	AM,AMC,CZ,RA,PRL,TE,FOX
7	1	AM,AMC,CZ,RA,PRL,FOX,CRO
8	1	AM,AMC,CZ,RA,PRL,CN,FOX,C
8	1	AM,AMC,CZ,RA,PRL,TE,FOX,TOP
8	1	AM,AMC,CZ,RA,PRL,CN,FOX,IPM
9	1	AM,AMC,CZ,RA,PRL,FEP,CN,FOX,C
9	1	AM,AMC,CZ,RA,PRL,CRO,CN,FOX,C
9	1	AM,AMC,CZ,RA,PRL,CFM,CN,FOX,DO
10	1	AM,AMC,CZ,RA,PRL,TE,CFM,CN,FOX,CRO
10	1	AM,AMC,CZ,RA,PRL,CFM,CN,FOX,CRO,IPM

(Table 7). Antimicrobial resistance Patterns of 9 Isolates of *E. coli* O157:H7 from Tap water in Basrah

Number of antibiotics	Number of isolation	Resistance Pattern
6	1	AM,AMC,CZ,RA,PRL,CN
6	1	AM,AMC,CZ,RA,PRL,IPM
8	1	AM,AMC,CZ,RA,PRL,TE,CN,IPM
8	1	AM,AMC,CZ,RA,PRL,CN,CFM,FOX
9	1	AM,AMC,CZ,RA,PRL ,CN,CFM,CRO,FOX
10	1	AM,AMC,CZ,RA,PRL,TE,CN,CFM,CIP,FOX
10	1	AM,AMC,CZ,RA,PRL,FEP,CN,CFM,IPM,FOX
10	1	AM,AMC,CZ,RA,PRL,FEP,ATM,CFM,CRO,FOX
11	1	AM,AMC,CZ,RA,PRL,TOP,DO,CFM,CRO,FOX,CN

4. Discussion

Although Enterohemorrhagic*Escherichia coli* OI57:H7has emerged as a pathogen of significant clinical importance to public health over the world. It is still missed during routine work of medical laboratories in Iraq. Data concerning to the presence of OI57:H7 in the environment in this area are lacking .

In the present study, high recovery of *E.coli* OI57:H7 isolates from sewage waste water indicate the needs of active treatment . Isolation of the bacterium from tap water is alarming which requiring improvement of water quality Isolation of the pathogen from river water is ordinary because the majority area of study ,people live in Villages work in upbreed of cattles and buffaloes which is resorvoir(source) of the dispersed of the bacterium from feacesthat take the way to the surface water .

4.1. Multidrug resistant E. coli OI7:H7 were

High number of patients in this rural area infected by this bacterium (10.3 %) towards (4.1 %) in urban area (Qurna city) (unpublished data) this may be attributed to the poor hygienic levels , direct contact with animals , uptake of raw milk without any treatment and swimming in rivers and ponds contaminated with feaces of cattle and buffalo .

A common features of the isolates from different sources in the present study is resistance to many antibiotics . These isolates considered to be multidrug resistance .

In a previous study, multidrug resistant *E. coli* are also isolated from patients with urinary tract infection in Basra [7] .Mutidrug resistant *E.coli*OI57:H7 arecommon obtained from farm animals and human infections [8]. It is apparent that these isolates are mostly resistant to beta-lactam antibioticswith low resistance to different fluoroquniolones. Multidrug Resistance in *E coli* 0157 were also isolated from surface waters in Nigeria [9].

MDR index was higher for isolates from sewage water followed by drinking water and tap water suggest that swage waste water environment may has an impact on this bacterium and the unptake of foreign DNA in these environments is high .

MDR index for clinical isolates was higher (0.8) than environmental isolates for the study area (Unpublished) .From the results, it appears that environmental isolates of *E.coli* OI57:H7 harboredone or more plasmids with molecular weight ranged from 1- >10 kb.

The results are agreed with the finding of [7] for the isolates of *E. coli*causing urinary tract infections . Itcan be concluded from the present results that the number of plasmids obtained is significantly correlated to the number of antibiotics that the bacteria resist . This leads to the suggestion that antibiotics resistant genes may carried on plasmids .

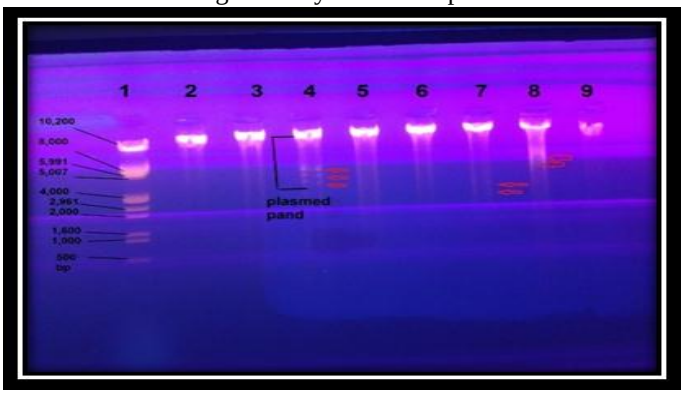

Figure 1. Plasmid profile of *Escherichia coli* 0157:H7 Strains fromsewage waste water in Basrah. Electrophoresed on 1% agrose gel. Lane-9 samples, L (1) DNA ladder.
L(2),L(3),L(5),L(6),L(9), showed One Plasmid
L(4), showed Four Plasmid
L(7), L(8), showed Three Plasmid

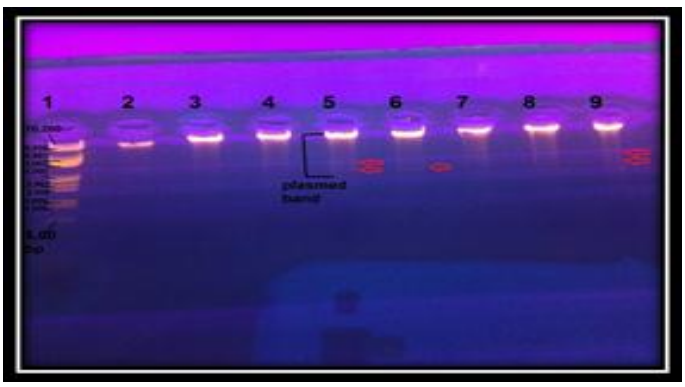

Figure 2. Plasmid profile of *Escherichia coli* 0157:H7 Strains from River water in Basrah. Electrophoresed on 1% agrose gel. Lane-9 samples,
L (1) DNA ladder.
L(2),L(3),L(4),L(7),L(8), showed One Plasmid
L(6), showed Two Plasmid
L(5), L(9), showed Three Plasmid

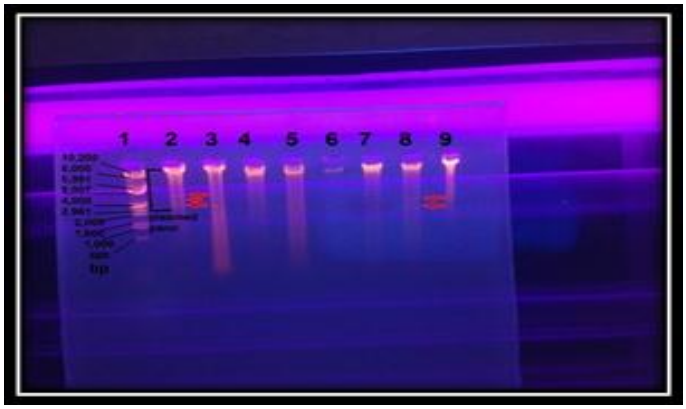

Figure 2. Plasmid profile of *Escherichia coli* 0157:H7 Strains from Tap water in Basrah. Electrophoresed on 1% agrose gel. Lane-9 samples, L (1) DNA ladder. L(3),L(4),L(5),L(6), L(7),L(9)showed One Plasmid L(2), showedFour PlasmidL(8), showed Three Plasmid

References:

[1] Brett, A. K. ,Hornitzky, M. A. , Bettelheim, K. A., Walker, M. J. and Djordjevic, S. P. (2003). Bovine non-O157 Shiga toxin 2-containing *Escherichia coli* possess stx2 - EDL933 and /or stx2 vhb Subtypes. J. Clin. Microbiol. 41: 2716-2722.

[2]Kaper, J.B. and O Brien A.D.(2014);Overview and historical perspectives.Microbielspectra. 2(2) :

[3]Khudaier , B. Y; Abbas, B. A; Khleel , K. A ;(2012)prevalence and antimicribial susceptibility of *Escherichia coli*O157:H7 isolated from human and animal sources in Basrah province.

[4]Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. (1966). Am. J. Clin .Pathol. 43:493–496.

[5]Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2012. (CLSI Document M 100-S22).

[6]Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res. 1979;7:1513–1523.

[7]Al-Hamdani ,M.A and Abbas, I.(2013); Study of Plasmid Profile and Susceptibility patterns of Escherichia coli isolated from patients with urinary tract infection in Basra.

[8]Aibinu, I.E; Peters, R.F; Amisu, K.O;Adesida, S.A; Ojo, M.O and Tolu, O. (2007) Multidrug Resistance in E.coli 0157 Strains and the Public Health Implication.

[9]Chigor , V. N;Umoh ,V. J; Smith, S. I ;Igbinosa, E.O. and Okoh, A. I.(2010) Multidrug Resistance and Plasmid Patterns of *Escherichia coli*O157 and Other *E. coli* Isolated from Diarrhoeal Stools and Surface Waters from Some Selected Sources in Zaria, Nigeria.