Phenotypic detection and antibiotics resistance pattern of local serotype of E. coli O157:H7 from children with acute diarrhea in Hilla city/ Iraq

Article *in* Journal of Pharmaceutical Sciences and Research · March 2018

CITATIONS
0

READS
34

3 authors, including:

Eiman Saeed
University of Basrah
18 PUBLICATIONS **3** CITATIONS

SEE PROFILE

Zainab H. Al-Saadi et al /J. Pharm. Sci. & Res. Vol. 10(3), 2018, 604-609

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Phenotypic detection and antibiotics resistance pattern of local serotype of *E. coli* O157:H7 from children with acute diarrhea in Hilla city/ Iraq.

Zainab H. Al-Saadi,* Adnan H. Tarish, Eiman A. Saeed***

**Department of Microbiology, College of Medicine, Babylon University*
***Pediatric Branch, College of Medicine, Babylon University*
Babylon, Iraq

Abstract:

The present study has been designed to isolate *E. coli* O157:H7 as an important zoonotic pathogen of food borne illness which often leads to diarrhea or hemorrhagic colitis in humans. *E. coli* O157:H7 is a major cause of hemolytic uremic syndrome that leads to acute renal failure in children. Stool samples were collected from 500 child (less than 5 years old) suffering from acute diarrhea (bloody, watery or both) and persistent diarrhea symptoms. First screening of *E. coli* was depend on culturing stool samples on Eosin Methylene Blue and MacConkey agar, then incubated aerobically at 37°C for 24hrs. Staining of single colony from the growth by Gram's stain was achieved. All samples were examined for the presence of *E. coli* O157:H7. Differentiation of bacteria from other non-sorbitol fermenting *E. coli* (NSF *E. coli*) by culturing the suspected bacteria on Sorbitol-MacConkey agar and *E. coli* O157:H7 Chromogenic Agar base with cefixime tellurite supplement. By using conventional biochemical tests (catalase, oxidase, and IMViC) without differentiation between serotype O157:H7 and other NSF *E. coli* isolates. Three specific biochemical tests (cellobiose fermentation, KCN and enterohemolycin production) were done to differentiate serotype O157:H7 from other NSF bacteria. Serological detection of O157 somatic antigen by using Slide agglutination of heat-treated organisms. For conformity identification of *E. coli* O157:H7, Vitek2 system were used. Sensitivity pattern of isolates from stool samples was done by antibiotics disc diffusion methods and results were recorded according to CLSI standards guidelines.

Results were exhibited that 114(22.8%) out of 500 were non-sorbitol fermenting *E. coli*. From 114 NSF *E. coli* isolates, only 11 Isolates were positive to *E. coli* O157:H7 at a percentage (2.2%). All isolates of *E. coli* O157:H7 gave positive results for Vitek2 system and rapid agglutination reaction. PCR positive isolates were produced enteroheomlysin on sheep blood agar. Multiple antibiotic resistances were observed and were found resistant to at least 13 of the 20 tested antibiotics. *E. coli* O157:H7 showed higher percentage of resistance 100% to (Ampicillin/Sulbactam, Cefepime, Cefotaxime, Ceftriaxone, Ceftazidime, Cephalothin, Aztreonam, Trimethoprim), 90.9% to (Amox/ Clavulanate, Nalidixic acid) and 81.8% to Azithromycin.

Keywords: *E. coli* O157:H7, Shiga-like toxins , Antibiotics susceptibility

1. INTRODUCTION:

E. coli O157:H7 is the major serotype belonging to the strain enterohemorrhagic *E. coli*. These strains have been found to be important food-borne pathogens (Huerta-Urebi *et al*., 2016; Kaneda *et al*., 2017).This type of *E. coli* can cause hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Jeong *et al*., 2010; De-Boer *et al*., 2015). *E. coli* O157:H7 is characterized by the expression of shiga-like toxins even though it produces various other virulence factors (Pianciola *et al*., 2016). Shiga toxins are classified into two major groups, Stx1 and Stx2, which are encoded on a prophage (Juma, 2010; Zhu *et al*., 2017). These genes can be transferred horizontally to *E. coli* (Basu and Tumer, 2015; Lindsey *et al*., 2016), allowing transformation of shiga-like toxin non-producing strains into shiga-like toxin-producing strains. **The aims** of this study was to isolate and identify local strains of *E. coli* O157:H7 which obtained from children with acute and persistent diarrhea, to characterize the strains by traditional phenotypic methods and specific biochemical tests and assess the antimicrobial susceptibility of these strains to obtain antibiotics resistance pattern.

2. MATERIAL AND METHODS:

2.1. Sampling

A total of 500 stool samples were collected from children under 5 years old with diarrhea from both sexes who attend to Babylon Maternity and Pediatrics Hospital in Babylon city, 211 females and 289 males.

Stool samples were collected from children they hospitalized with acute diarrhea (bloody, watery or both) and persistent diarrhea symptoms during the period from July 2016 to February 2017. A detailed history of the patients is obtained, including information on the age, sex, source of drinking water, type of feeding and antibiotics usage.

2.2 Isolation of *E. coli* O157:H7

The fecal swabs were collected by using sterile disposable wooden swabs, they were inoculated into MacConkey agar and incubated aerobically at 37ºC for 24hrs. Rose pink coloured colonies with typical appearance of *E. coli* were re-streaked on Eosin Methylene Blue agar, then incubated aerobically for 24hrs., at 37°C. The green metallic sheen colonies were considered as *E. coli*. Morphological identification completed by Gram's staining technique according to (CDC, 2009; Paresh *et al.*, 2013).

E. coli positive colonies should be transferred to another medium for subsequent testing for *E. coli* O157:H7, Sorbitol MacConkey agar – SMAC (Himedia) was used as selective medium, suspected colonies were inoculated , then incubated aerobically at 37°C for 24hrs., pale or amber colour colonies were appeared (sorbitol non-fermenting colonies).

Sorbitol negative colonies were re-cultured on MacConkey agar medium to eliminate non lactose fermenting bacterial species, incubated at 37°C for 24hrs., then colonies transferred on *E. coli* O157:H7 Chromogenic Agar base with cefixime tellurite supplement, for first screening for *E. coli* O157:H7 incubated at 37ºC for 24hrs. pale pink to faint red color colonies were appeared.

2.2.1. Slide agglutination of heat-treated organisms:

Serological detection of O157 somatic antigen by using slide agglutination of heat-treated organisms were done. This test was used for more specific identification of *E. coli* O157:H7 by using commercial reagent (pathogenic *E. coli* "O" Antisera, Mast Assure™). The test performed according to the instructions of the manufacturing company (Mast Group Ltd.) described with modifications as follows:

- A dense suspension of *E. coli* 0157:H7 was prepared by taking 3-5 match head size amounts of 24hrs. growth of tested bacteria suspended in 3ml of 0.85% normal saline.
- The suspension was Autoclaved at 121°C for 15min. and centrifuged at 900g for 20 min.
- The supernatant should then be removed , 0.5ml of 0.85% normal saline was added to re-suspend the precipitate.
- The suspension was homogenized by mixing, then used as antigenic suspension for O-antigen grouping.
- Two loopfuls or drops (5-10µl) of antigenic suspension were placed onto clean microscope slide. The slide partitioned by using china graph pencil.
- A drop of polyvalent antiserum was placed onto one of the drops of emulsified isolate. Control made by adding of a drop of polyvalent antiserum onto drop of 0.85% normal saline.
- The reagents mixed by tilting the slide back and forth for 60 sec. while viewing it under indirect light against a dark background.
- Distinct clumping or agglutination within this period, without clumping in the saline control (autoagglutination), should be regarded as a positive result. Weak agglutination should be recorded as negative.

2.2.2. Specific biochemical tests for *E. coli* O157:H7 (Cellobiose fermentation test, Potassium Cyanide growth test, and Enterohemolysin production) were performed on the non-sorbitol fermenting colonies (Sanderson *et al*., 1995; Stapp *et al*., 2000), and **Vitek2 system** were used for conformity identification of *E. coli* O157:H7.

2.2.3. Identification process with Vitek2 System:
In clinical microbiology Vitek2 system used as an auto instrument system for the identification (ID) and antibiotic susceptibility testing (AST). The samples were achieved according to manufacture instructions as follows: a sterile plastic stick applier used to take pure colonies from culture media and transfer a sufficient number of them to plastic test tubes.
All reagent and instrumentation required for process provided by Manufacturer Company. All isolates introduced to the computer before process and inoculated cards were processed within the instrument within 30 min of inoculation.
GP (Gram positive) cards were loaded (inoculated) with bacterial suspensions employing a vacuum chamber in machine. Check tubes containing the samples were placed into a cassette (special test tube rack) and therefore the identification card was placed within the neighboring place where as inserting the transfer tube into the corresponding suspension tube.
The cassette might accommodate up to 10 test tubes. The filled cassette was placed into a vacuum chamber station inside the Vitek 2 instrument machine.
The vacuum was applied then the air was recharged into the station, the bacterial suspension was forced through the transfer tube into micro channels that filled all the test wells. Inoculated cards were passed by a mechanism, that stop the transfer tube and sealed the card before loading into the circular incubator. The incubator might accommodate up to 30 cards.
All card varieties were incubated at 35.5±1ºC. every card was removed from the incubator once each 15 min., transported to the optical system for reaction readings, so came to the incubator until future read time information were collected at 15 min. intervals throughout the whole incubation period.

2.2.4. Antibiotics susceptibility testing:
Antibiotic susceptibility test was performed by disc diffusion method to measure the patterns of antibiotics resistance among the isolates by using the disc diffusion method and Mueller – Hinton agar as recommended by the Clinical and Laboratory Standard Institute guidelines (CLSI, 2016) using commercially available antibiotics discs. Twenty type of antibiotics were used to test *E. coli* O157:H7and their respective concentrations (table 1).
By using Muller-Hinton agar medium, inoculated with 10^6CFU/ml bacterial suspension (according to McFarland standard scale), the antibiotic discs were placed on the inoculated plate, incubated at 37ºC for 24 hrs., then the diameter of inhibition zones were measured. The inhibition zones were measured and interpreted as sensitive, intermediate, or resistant in accordance with (CLSI, 2016) criteria.

Table (1): Antibiotics types used in Antibiotics Susceptibility Test

Antibiotic	Disk contents (µg)	Susceptible	Intermediate	Resistant
Ampicillin/Sulbactam (SAM)	(10/10) µg	≥ 15	12 – 14	≤ 11
Amox/Clavu (AUG)	(20/10) µg	≥ 18	14 – 17	≤ 13
Cefepime (FEP)	30 µg	≥ 25	19 – 24	≤ 18
Cefotaxime (CTX)	30 µg	≥ 26	23 – 25	≤ 22
Ceftriaxone (CRO)	30 µg	≥ 23	20 – 22	≤ 19
Cefoxitin (FOX)	30 µg	≥ 18	15 – 17	≤ 14
Ceftazidime (CAZ)	30 µg	≥ 21	18 – 20	≤ 17
Cephalothin (KF)	30 µg	≥ 15	_	≤ 14
Aztreonam (ATM)	30 µg	≥ 21	18 – 20	≤ 17
Imipenem (IMI)	10 µg	≥ 23	20 – 22	≤ 19
Meropenem (MEM)	10 µg	≥ 23	20 – 22	≤ 19
Gentamicin (GM)	10 µg	≥ 15	13 – 14	≤ 12
Amikacin (AK)	30 µg	≥ 17	15 – 16	≤ 14
Azithromycin (ATH)	15 µg	≥ 13	_	≤ 12
Tetracycline (TE)	30 µg	≥ 15	12 – 14	≤ 11
Ciprofloxacin (CIP)	5 µg	≥ 21	16 – 20	≤ 15
Nalidixic acid (NA)	30 µg	≥ 19	14 – 18	≤ 13
Trimethoprim (TM)	5 µg	≥ 16	11 – 15	≤ 10
Chloramphenicol (C)	10 µg	≥ 18	13 – 17	≤ 12
Nitrofurantoin (F)	300 µg	≥ 17	15 – 16	≤ 14

3. RESULTS AND DISCUSSION

3.1. Prevalence of *E. coli* O157:H7:
During the present investigation, results revealed that 114(22.8%) out of 500 stool samples were identified as non-sorbitol fermenting (NSF) *E. coli*. From 114 NSF *E. coli* isolates, only 11(2.2%) Isolates were *E. coli* O157:H7.

3.2 Identification of *E. coli*:
The identification of *E. coli* depends on the cultural characteristics, microscopic patterns, biochemical tests, and Vitec2 system.

3.2.1 The Characterization of *E. coli* isolates on culture media:

On MacConkey agar medium the colonies of *E. coli*, appeared red pink color due to the utilizing the lactose available in the medium with surrounding areas of precipitated bile salts, by utilizing the lactose available in the medium, Lactose fermenting bacteria such as *E. coli* will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of pink colonies. The bile salts precipitate in the immediate neighbourhood of the colony, causing the medium surrounding the colony to become hazy (Quinn *et al*., 2004; Johnson, 2013).

On Eosin Methylene Blue agar the colonies appeared as green metallic sheen, EMB agar it is commonly used as both a selective and a differential medium. Eosin-methylene blue agar is selective for gram-negative bacteria against gram-positive bacteria. In addition, EMB agar is useful in isolation and differentiation of the various gram-negative bacilli and enteric bacilli, The bacteria which ferment lactose in the medium form colored colonies, while those that do not ferment lactose appear as colorless colonies, *Escherichia coli* colonies grow with a metallic sheen with a dark center, Aerobacter aerogenes colonies have a brown center, and non-lactose-fermenting gram-negative bacteria appear pink. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria (Quinn *et al*., 2004; Madigan *et al*., 2006). Eosin is a dye that responds to changes in pH, going from colorless to black under acidic conditions. EMB agar medium contains lactose and sucrose, but not glucose, as energy sources. The sugars found in the medium are fermentable substrates which encourage growth of some gram-negative bacteria, especially fecal and non-fecal coliforms. Differentiation of enteric bacteria is possible due to the presence of the sugars lactose and sucrose in the EMB agar and the ability of certain bacteria to ferment lactose in the medium. Lactose-fermenting Gram negative bacteria (generally enteric) acidify the medium, and under acidic conditions the dyes produce a dark purple complex which is usually associated with a green metallic sheen. This metallic green sheen is an indicator of vigorous lactose and/or sucrose fermentation ability typical of fecal coliforms. A smaller amount of acid production, which is a result of slow fermentation (by slow lactose-fermenting organisms), gives a brown-pink coloration of growth. Colonies of non-lactose fermenters appear as translucent or pink (Leininger *et al*., 2001).

Under light microscope, the isolated bacteria appeared as Gram negative, rod shaped, non-spore forming bacteria.

3.2.2 Biochemical tests for *E. coli*:

E. coli colonies gave positive results for biochemical tests (Catalase, Oxidase, and IMViC tests) (table 2).

Table (2). Results of some biochemical tests for *E. coli*

Biochemical tests	Results
Catalase	+
Oxidase	_
Indole production	+
Methyl Red	+
Vokes-Proskauer	_
Citrate utilization	_

3.3 Specific confirmation of *E. coli O157:H7*:

3.3.1 Culturing on Sorbitol MacConkey agar medium (SMAC).

The results of growth on Sorbitol MacConkey agar appeared as colorless or amber like colonies that referred as non sorbitol fermenting isolates (NSF), while the other types of *E. coli* appeared with pink color colonies (sorbitol fermenting _ SF). Sorbitol MacConkey agar was a variant of traditional MacConkey agar used in the detection of *E. coli O157:H7* and useful in screening for it in fecal specimens. *E. coli O157:H7* that was differ from most other strains of *E. coli* in being unable to ferment sorbitol. In Sorbitol MacConkey agar, lactose replaced by sorbitol. Most strains of *E. coli* ferment sorbitol to produced acid, but *E. coli O157:H7* cannot ferment sorbitol, so can differentiate *E. coli O157:H7* from other strains of *E. coli* depending on the fact that *E. coli O157:H7* unlike 90% of *E. coli* isolates does not ferment sorbitol (March and Ratnam, 1986; Novicki *et al*., 2000) .

3.3.2 Culturing on *E. coli O157:H7* Chromogenic agar base with cefixime tellurite supplement:

The results of growth on *E. coli O157:H7* Chromogenic agar base with cefixime tellurite supplement were exhibited pale pink to faint red color colonies. **Chromogenic agar** media have more advantage and can be an appropriate alternative for conventional and routine procedure. Chromogenic media eliminate the need of subculture and further biochemical test for identification pathogenic agent and at the shortest period of time pathogenic agent can be identified. These media are very specific and their component act as substrate for specific enzyme and depending on enzyme exhibit special color (Tavakoli *et al*., 2008; Ngwa *et al*., 2013), peptone mixture provides nitrogens, vitamins, minerals and amino acids essential for growth. Chromogenic mixture allows to easy detect the presence of *E. coli O157:H7* by colony coloration that grows pale pink. Potassium tellurite and cefixime were highly selective for *E. coli O157:H7* and inhibit most contaminating bacteria including other *E. coli* strains and coliforms (Doyle and Choeni, 1987), in addition, resistant of *E. coli O157:H7* for tellurite and therefore grows in concentration often that inhibit most other *E. coli* strains (Orth *et al*., 2007).

3.3.3 Slide agglutination of heat-treated organisms (detection of *E. coli* O157 antigen):

Results of the reaction between *E. coli* antigenic suspension (emulsified isolates) and polyvalent antisera were revealed distinct agglutination (granular clumping) within a 60 seconds of reaction, there is no agglutination in the saline control, which mean positive results and an indicator for the presence of *E. coli* O157. Isolates producing a distinct positive reaction with a polyvalent antiserum are assumed to be an *E. coli* bearing one or more of the O antigenic factors represented by that antiserum. Further testing of the isolates should be done for more confirmation. According to CDC, (2015) Specimens from which sorbitol-negative colonies have been isolated that agglutinate in O157 antiserum or O157 latex reagent, and are biochemically *E. coli*, may be reported as presumptively positive for *E. coli O157:H7*, isolates agglutinating in O157 antiserum or O157 latex reagent should be identified biochemically as *E. coli*, since strains of several species cross-react with O157 antiserum. Sowers *et al*., (1996) were found that Sorbitol-non-fermenting colonies from selective media must be identified as *E. coli O157*. This is conveniently done using slide agglutination with specific antiserum or commercially available anti-O157 latex reagents and standard biochemical tests (Vuddhakul *et al*., 2000).

3.3.3 Specific biochemical tests for *E. coli* O157:H7:

The results of specific biochemical tests were showed that *E. coli* O157:H7:

(1): cannot ferment cellobiose sugar (cellobiose fermentation --)

(2): cannot grow in the presence of cyanide potassium (KCN --)

(3): produce enterohemolysin on sheep blood agar (enterohemolysin production +)

For more definitive identification of *E. coli* O157:H7 there is a specific biochemical tests that can be used to differentiate which is closely related with it metabolites.

All tested isolates of *E. coli* O157:H7 have negative results for cellobiose fermentation and the growing in the presence of potassium cyanide. *E. coli* O157:H7 cultures that obtained from

fecal samples was failed to attack cellobiose. Culturing of *E. coli* O157:H7 in potassium cyanide broth did not showed any turbidity (Hitchins *et al.*, 1998). *E. hermanni* was biochemically similar to *E. coli* O157:H7 (in non-sorbitol fermentation character), but can distinguished it by cellobiose fermentation and its ability to grow in potassium cyanide, therefore the main biochemical tests that required to distinguish between them was growing in the presence of potassium cyanide and cellobiose fermentation (Al-Dawmy and Yousif, 2013).

The third confirmatory test was enterohemolysin production, *E. coli* O157:H7 isolates were showed their ability to produce enterohemolysin when they grew on washing sheep blood agar, hemolysis appeared as a small, turbid, unclear regions around colonies (Bettelheim, 2003, Gould *et al.*, 2009). Enterohemolysin, a pore-form-ing toxin, induces the production of IL-1ß, which is one of serum risk markers for HUS (Taneike, 2002).

3.3.4 Identification of *E. coli* O157:H7 by Vitek 2 system:

The final identification was performed with the automated VITEC@2 system using GN-ID cards which contained (64) biochemical tests Table (2). The results demonstrate that all (11) isolates for *E. coli* O157:H7 were confirmed with ID message confidence level ranging excellent (Probability percentage from 93 to 98%). This technique is characterized by fast detection of bacteria without need for many of culture media as well as reduces cultures contamination.

3.4 Antibiotics susceptibility:

Results of antibiotics susceptibility test were exhibited that *E. coli* O157:H7 resistant to at least 13 of the 20 antibiotics tested, showed higher percentage of resistance 100% to (Ampicillin/Sulbactam, Cefepime, Cefotaxime, Ceftriaxone, Ceftazidime, Cephalothin, Aztreonam, Trimethoprim), 90.9% to (Amox/ Clavulanate, Nalidixic acid) and 81.8% to Azithromycin. The lowest level of resistance was observed in Imipenem, Amikacin, and Chloramphenicol (36.4%).

Also, results revealed a varying degrees of susceptibility to antibiotics tested, high percentage of *E. coli* O157:H7 isolates were sensitive to amikacin , azthromycin, gentamicin, and tetracyclin.

Chloramphenicol, ciprofloxacin, and imipenem were revealed intermediate action against the isolates. Table (3) and (4) illustrates the susceptibility pattern of 11 *E. coli* O157:H7 isolated strains from different hospitals against the regular used antibiotics.

This table shows the distribution of individual isolate with of *E. coli* O157:H7 according to antibiotic resistance. The first isolate is the most resistant one for antibiotics (20 types).

Rate of multidrug resistance in this study is in concordance with reports which have documented high resistance in *E. coli* causing diarrhea infections in children, especially *E. coli O157:H7* (Thapar and Sanderson, 2004; Sudershan *et al.*, 2014). A total of the 11 isolates obtained in this study were resistant to at least 13 of the antibiotics used, with highest resistance rates observed against the 100% to (Ampicillin/Sulbactam, Cefepime, Cefotaxime, Ceftriaxone, Ceftazidime, Cephalothin, Aztreonam, Trimethoprim), 90.9% to (Amox/ Clavulanate, Nalidixic acid) and 81.8% to Azithromycin.

The obtained results in this study were compatible with the results of (Hassan, 2015), which concluded that the high-incidence rate of *E. coli* O157:H7 infection in children associated with limited number of drugs effective against *E. coli* O157:H7 with high prevalence of resistance to more than three antibiotics. Egbule *et al.*, (2016) found that Serotypes O157 exhibited 100% resistance to ceftazidime, cefuroxime and cefixime. The ability of *E. coli* O157 strains to transfer antimicrobial resistance traits by conjugation, high level of resistance transferred was observed. Also, they observed the ease of transfer exhibited by *E. coli* O157:H7 strains amongst children is an issue of concern.

Table (3): Antibiotic susceptibility pattern of the isolated strains *E. coli* O157:H7

Antibiotics	Antibiotic sensitivity			Total
	Sensitive	Intermediate	Resistance	
Amp/Sulba	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Amox/ Clavu	0 (0.0%)	1 (9.1%)	10 (90.9%)	11 (100.0%)
Cefepime	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Cefotaxime	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Ceftriaxone	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Cefoxitin	2 (18.2%)	2 (18.2%)	7 (63.6%)	11 (100.0%)
Ceftazidime	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Cephalothin	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Aztreonam	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Imipenem	3 (27.2%)	4 (36.4%)	4 (36.4%)	11 (100.0%)
Meropenem	1 (9.1%)	3 (27.2%)	7 (63.6%)	11 (100.0%)
Gentamicin	5 (45.5%)	0 (0.0%)	6 (54.5%)	11 (100.0%)
Amikacin	4 (36.4%)	3 (27.2%)	4 (36.4%)	11 (100.0%)
Azithromycin	2 (18.2%)	0 (0.0%)	9 (81.8)	11 (100.0%)
Tetracycline	6 (54.5%)	0 (0.0%)	5 (45.5%)	11 (100.0%)
Ciprofloxacin	1 (9.1%)	2 (18.2%)	8 (72.7%)	11 (100.0%)
Nalidixic acid	0 (0.0%)	1 (9.1%)	10 (90.9%)	11 (100.0%)
Trimethoprim	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (100.0%)
Chloramphenicol	2 (18.2%)	5 (45.5%)	4 (36.4%)	11 (100.0%)
Nitrofurantoin	0 (0.0%)	2 (18.2%)	9 (81.8%)	11 (100.0%)

Table (4): Antibiogram of individual isolates of *E. coli* O157:H7

Isolate number	Antibiotic resistance	
	Number	Name
First	20	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, IMI, MEM, GM, AK, ATH, TE, CIP, NA, TM, C, F
Second	15	SAM, AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, IMI, MEM, CIP, NA, TM, F
Third	16	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, MEM, TE, CIP, NA, TM, C, F
Fourth	18	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, MEM, GM, AK, ATH, TE, CIP, NA, TM, F
Fifth	14	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, ATH, CIP, NA, TM, F
Sixth	17	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, IMI, MEM, ATH, TE, CIP, NA, TM, F
Seventh	14	SAM , AUG, FEP, CTX, CRO, CAZ, KF, ATM, GM, ATH, CIP, NA, TM, F
Eighth	14	SAM , AUG, FEP, CTX, CRO, CAZ, KF, ATM, MEM, ATH, NA, TM, C, F
Ninth	15	SAM , FEP, CTX, CRO, CAZ, KF, ATM, GM, ATH, TE, CIP, NA, TM, C, F
tenth	13	SAM, AUG, FEP, CTX, CRO, CAZ, KF, ATM, GM, AK, ATH, NA, TM
Eleventh	15	SAM , AUG, FEP, CTX, CRO, FOX, CAZ, KF, ATM, IMI, MEM, GM, AK, ATH, TM,

Al-Jarousha, 2015 demonstrated in his investigation that *E. coli* O157:H7 isolated from children with diarrhea reflects variable susceptibility with highest resistance to ampicillin, trimethoprim/sulphamethoxazole and Tetracycline, in these results demonstrated that *E. coli* O157 expressed resistant to most empirical antibiotics used in their control and need to be reviewed by treating physicians. Much of the reasons for these high rates of resistance are related to the fact that reports have shown that antibiotics, despite not being required for the treatment of acute diarrhea, are widely prescribed for these forms of infections (Isibor and Ekundayo, 2012). In children, this is made worse as cheap drugs are available over the counter and the wide majority of parents are unaware that antibiotics rarely alter the course of diarrheal infections and so administer one form of antibiotic or the other to their wards whenever a diarrheal infection is suspected. The continued use and abuse of these drugs thus allows for the selection of resistant strains which are easily disseminated (Munoz-Davila, 2014). At this point, education of the masses on the management of diarrheal infections as well as the implementation of more stringent policies governing the availability of antibiotics is advised (Olorunshol *et al*., 2000).
In present study, results also revealed varying degrees of susceptibility to antibiotics tested, high percentage of *E. coli* O157:H7 isolates were sensitive to amikacin, azthromycin, gentamicin, and tetracyclin, while chloramphenicol, ciprofloxacin, and imipenem were revealed intermediate action against the isolates, the gained results were compatible with results of Hassan, (2015) in which *E. coli* O157: H7 was showed moderate-to-low rate of resistance to ciprofloxacin, amikacine, ceftriaxone and Imipenem.

Islam *et al*., 2016 were proved that highest susceptibility of the *E. coli* isolates was found against Gentamicin (92.59%), followed by ciprofloxacin (48.14%) and Moxifloxacin (33.33%). Gentamicin and ciprofloxacin can be recommended as the effective drugs successful treatment of STEC infections in children.

Ayatollahi *et al*., 2013 found in their study to investigate antimicrobial-resistance of *Escherichia coli* isolated from children in Shahid Sadoughi hospital of Yazd, that Ciprofloxacin was the most active antibacterial agent (78% susceptible), followed by gentamicin. The previous recorded results were compatible with our results in pattern of sensitivity. Also, Khudaier *et al*., (2012) were revealed the antibiotics susceptibility pattern of E. coli O157:H7 in their study, that sensitive to gentamicin and amikacin, these results were correspond with our results.

CONCLUSION:

This study revealed that *E. coli* O157:H7 isolated from children under five years old, diagnosed and identified by using Sorbitol MacConkey agar and *E. coli* O157:H7 Chromogenic Agar supplemented with cefixime tellurite which considered as a good tool for first screening of this pathogen. Specific biochemical tests for *E. coli* O157:H7 (cellobiose fermentation test, KCN production, and enterohemolysin production) were achieved to accomplish the identification. The antibiotics susceptibility pattern reflects variable susceptibility with highest percentage of resistance to Ampicillin/Sulbactam, Cefepime, Cefotaxime, Ceftriaxone, Ceftazidime, Cephalothin, Aztreonam, Trimethoprim.

Antibiotics treatment decreases the chance of recovery of *E. coli* O157:H7, therefore, when follow-up specimens are being obtained, the patient should have received no antibiotic for a minimum of 48 hours before culture.

REFERENCES:

1. Al –Dawmy, F. A. and Yousif, A. A. (2013). Prevalence of *E. coli O157:H7* in intestinal and Urinary tract infection in children. Intern. J Adv. Res.1(8): 111-120.
2. Al_Jarousha, A. KH. (2015). The Epidemiology and Antibiotic Resistant Pattern of enterohemorrhagic *E. coli* O157 in the stool of Diarrheal children in Gaza Strip. Annals of Alquds Medicine., 11(1436):2-16.
3. Ayatollahi, J. ; Shahcheraghi, S. H. ; Akhondi, R. ; Soluti, S. S. (2013). Antibiotic Resistance Patterns of *Escherichia coli* Isolated from Children in Shahid Sadoughi Hospital of Yazd. Iranian Journal of Pediatric Hematology Oncology., 3(2):78-82.
4. Basu, D. and Tumer, N. E. (2015). Do the A Subunits Contribute to the Differences in the Toxicity of Shiga Toxin 1 and Shiga Toxin 2?. Toxin, 7:1467-1485.
5. Bettelheim, K. A. and Beutin, L. (2003). Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). J App. Microbiol., 95:205-217.
6. CDC- Centers for disease control and prevention. (2009). Recommendations for Diagnosis of Shiga Toxin–Producing *Escherichia coli* Infections by Clinical Laboratories. Vol. 58 / RR-12.
7. CSLI_ Clinical and laboratory standard institute (2016). Performance Standards for Antimicrobial Susceptibility Testing. M100S, 26th ed. Wayne, Pennsylvania,USA.
8. De Boer, F. R.; Ferdous, M.; Ott, A. ; Scheper, R. H.; Wisselink, G. J.; Heck, E. M.; Rossen, W. J.; Kooistra-Smid, A. M. D. (2015). Assessing the Public Health risk of shiga toxin-producing *Escherichia coli* by use of a rapid diagnostic screening algorithm. JCM, 53(5):1588-1598.
9. Doyle, M.P., and J.L. Schoeni. 1987. Isolation of Escherichia coli O157:H7 from retail meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.
10. Egbule, O. S.; Owhe-Ureghe, U. B.; and Odih, E. E. (2016). Occurrence of Multidrug Resistance among *E. coli O157:H7* Isolated from Stool Samples Obtained from Hospitalized Children. J Prob Health., 4(3):1-4.
11. Gould, L. H. ; Bopp, CH.; Strockbine, N.; Atkinson, R.; Baselski, V.; Body. Carey, R.; Crandall, C.; Hurd, SH.; Kaplan, R.; Neill, M.; Shea, SH.; Somsel, P.; Tobin-D'Angelo, M.; Griffin, P.; and Gerner-Smidt, P. (2009). Recommendations for Diagnosis of Shiga Toxin--Producing *Escherichia coli* Infections by Clinical Laboratories. MMWR-CDC, 58(RR12);1-14.
12. Hassan, J. S. (2015). Antimicrobial Resistance Patterns of *Escherichia coli* O157:H7 Isolated from Stool Sample of Children. Iraqi JMS 13: 259-264.
13. Hitchins, A. D., Feng, P., Watkins, W. D., Rippey, S. R., and Chandler, L. A. 1998. *Escherichia coli* and the coliform bacteria. Ch4. In Food and Drug Administration Bacteriological Analytical Manual, 8th ed.
14. Huerta-Uribe, A.; Margenberg, R. Z.; Yamaguchi, N.; Fitzgerald, S.; Connolly, J. P. R.; Carpena, N.; Uvell, H.; Douce, G.; Elofsson, M.; Byron, O.; Marquez, R.; Gally, L. D.; and Roe, A. J. (2016). Identification and characterization of novel compounds blocking shiga toxin expression in *Escherichia coli* O157:H7. Frontiers Microb., 7:1-9.
15. Isibor, J. O. and Ekundayo, A. O. (2012). Determination of the antibiotic susceptibility patterns of local isolates of *E. coli O157:H7* from Edo state, Nigeria. New york Sci. J., 5:151-157.
16. Islam, M. M. ; Ahamed, S. ; Arafat, M. Y.; Hasan, I. ; Rahman, M. ; and Nazir, K. H. M. N. H. (2016). Molecular detection and antibiogram of shiga toxin producing Escherichia coli (STEC) isolated from diarrheic children. Bangl. J. Vet. Med., 14 (2): 289-295.
17. Jeong, K.; Tzipori, S.; and Sheoran, S. A. (2010). Shiga Toxin 2–Specific but Not Shiga Toxin 1–Specific Human Monoclonal Antibody Protects Piglets Challenged with Enterohemorrhagic *Escherichia coli* Producing Shiga Toxin 1 and Shiga Toxin 2. JID, 201:1081–1083.
18. Johnson, T. R.; Case, Ch. L.; Cappuccino, J. G.; Sherman, N. (2013). Great Adventures In The Microbiology Laboratory. Microbiology 22, 7th ed., 175-176.
19. Juma, A. S. M. (2010). Genetic transformation in antibiotics resistant *Escherichia coli* O157:H7. Med J Islamic World Acad Sci., 18(2):75-84.
20. Kaneda, H.; Shimizu, M.; and Yachie, A. (2017). Successful Treatment of Enterohemorrhagic *Escherichia coli*-Induced Acute Encephalopathy and Hemolytic-Uremic Syndrome With Polymyxin-B Direct Hemoperfusion. J Am Soc. Nephrol.
21. Bassam Y. Khudaier, Basil A. Abbas, Khulood A. Khleel. (2012). Prevalence and antimicrobial susceptibility of Escherichia coli O157:H7 isolated from human and animal sources in Basrah province. Bas.J.Vet.Res., 11(2):47-55.
22. Leininger, D. J.; Roberson, J. R.; and Elvinger, F. (2001). Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. J Vet Diagn Invest 13:273–275.
23. Lindsey, L. R.; Pouseele, H.; Chen, C. J.; Strockbine, A. N.; Carleton, A. H.(2016). Implementation of whole genome sequencing (WGS) for identification and characterization of shiga toxin-producing *Escherichia coli* (STEC) in the united states. Front. Microbiol., 7(766):1-9.
24. Madigan, M. T.; Martinko, J. M.; and parker, J. (2006). Brock biology of microorganisms: an international edition, 11th ed. Prentice-Hall international, Inc., Upper Saddle River, NJ.
25. March, S. M. and Ratnam, S. (1986). Sorbitol-MacConkey Medium for Detection of *Escherichia coli O157:H7* Associated with Hemorrhagic Colitis. J. Clin. Microbiol., 23(5): 869-872.
26. Munoz-Davila, M. J. (2014). Role of old antibiotics in the era of antibiotics resistance. Highlighted Nitrofurantoin for the treatment of lower urinary tract infections. Antibiotics, 3:39-48.

27. Ngwa, G. A. ; Schop, R. ; Weir, S. ; León-Velarde, C. G. ; and Odumeru, J. A. (2013). Detection and enumeration of *E. coli* O157:H7 in water samples by culture and molecular methods. J. Microbiol. Meth., 92:164-172.
28. Novicki, T. J.; Daly, J. A.; Mottice, S. L.; and Carroll, K. C. (2000). Comparison of Sorbitol MacConkey Agar and a two-step method which utilizes enzyme-linked immunosorbent assay toxin testing and chromogenic agar to detect and isolate enterohemorrhagic *Escherichia coli*. J. Clin. Microbiol., 38(2):547-551.
29. Olorunshol, I. D.; Smith, S. I.; and Coker, A. O. (2000). Prevalence of EHEC O157:H7 in patients with diarrhea in Logas, Nigeria. Acta Pathologica Microbiologica immunologica Scandinavia (APMIS). 108:761-763.
30. Orth, D. ; Grif, K.; Dierich, M. P.; and Wu¨rzner, R. (2007). Variability in tellurite resistance and the ter gene cluster among Shiga toxin-producing *Escherichia coli* isolated from humans, animals and food. Res. Microbiol., 158 : 105-111.
31. Paresh K. Virpari, J. B. Nayak, H. C. Thaker, M. N. Brahmbhatt. (2013). Isolation of pathogenic Escherichia coli from stool samples of diarrheal patients with history of raw milk consumption. Vet. Worl., 6(9):659-663.
32. Pianciola, L.; D'Astek, B. A. Mazzeo, M.; Chinen, I.; and Rivas, M. M. (2016). Genetic features of human and bovine *Escherichia coli* O157:H7 strains isolated in Argentina. IJMM, 1-23.
33. Quinn, P. J. ; Carter, M. E.; Markey, B. and Carter, G. R. (2004): Clinical Veterinary microbiology. 6th ed. Mosby an imp. Wolf , London., Pp: 66 – 85.
34. Sanderson, M. W.; Gay, J. M.; Hancock, D. D.; Gay, C. C.; Fox, L. K. ; Besser, T. E. (1995). Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J. Clin. Microbiol., 33:616- 619.
35. Sowers, E. G.; Wells, J. G.; and Strockbine, N. A. (1996) Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli*. J. Clin. Microbiol., 34:1286– 1289.
36. Sudershan, R.V. ; Kumar, R. N.; Kulkarni, B.; Kashinath, L.; Bhaskar, V.; and Polasa, K. (2014). *E. coli* pathotypes and their antibiotic resistance in young children with diarrhea in Hyderabad, India. Int. J. Curr. Microbiol. App. Sci., 3(9): 647-654.
37. Taneike, I.; Zhang, H.; Wakisaka-Saito, N.; and Yamamoto, T. (2002). Enterohemolysin operon of Shiga toxin-producing *Escherichia coli*: a virulence function of inflammatory cytokine production from human monocytes. FEBS Letters 524 : 219-224.
38. Tavakoli, H; Bayat, M; Kousha, A and Panahi. P. (2008): The Application of Chromogenic Culture Media for Rapid Detection of Food and Water Borne Pathogen. American-Eurasian J. Agric. & Environ. Sci., 4 (6): 693-698.
39. Thapar, N, and Sunderson, I. R. (2004). Diarrhea in children: an interface between developing and developed countries. Lancet., 363:641-653.
40. Vuddhakul, V.; Patararungrong, N.; Pungrasamee, P.; Jitsurong, S.; Morigaki, T.; Asai, N.; and Nishibuchi, M. (2000). Isolation and characterization of *Escherichia coli* O157 from retail beef and bovine feces in Thailand. *FEMS Microbiology Letters*, 182(2): 343–347.
41. Zhu, Ch.; Setty, P.; Boedeker, C. E. (2017). Development of live attenuated bacterial vaccines targeting *Escherichia coli* heat-labile and heat-stable enterotoxins. Vet. Microbiol., 202:72-78.