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RESEARCH ARTICLE

Prevalence of *E.coli* O157:H7 in intestinal and Urinary tract infection in childrenFaten A,A, Al –Dawmy¹ and *Afaf,Abdulrahman Yousif²

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Abstract

This study was conducted to isolate *E.coli* O157: H7 as an important zoonotic pathogen from 230 children suffering from diarrhea and 200 children had complication related to urinary tract infection (UTI) in Pediatric Hospital, in both cases children was less than 10 years.

Diagnostic study depends on culturing of each sample on MacConkey agar and Eosin Methylene blue agar then incubated aerobically at 37 C° for 24 hours. Staining of single colony from the growth by gram stain, biochemical tests (Kligler Iron medium (KI), Ureas test, Indole test, Motility, Citrate utilization test) were done on isolated bacteria to confirm diagnosis of *E.coli*. The isolated *E.coli* on MacConkey was cultured on Sorbitol MacConkey agar plus cefixime potassium tellurite (SMA-CT) and Chrom agar™ *E.coli* O157:H7 and incubated at 37 C° for 24 hrs in addition to using of Potassium Cyanide (KCN) Test as specific biochemical test. Latex agglutination test was used for serotyping of *E.coli* O157: H7.

The results revealed that 126 out of 230 were positive to *E.coli*. From 126 *E.coli* only 11 isolates were positive to *E.coli* O157:H7 at a percentage (4.78%). The number of *E. coli* isolates in urine samples were 98 from 200 samples, only 3 isolates gave positive results to *E.coli* O157:H7 at a percentage (1.5%). According to age effect, the study revealed that most diarrheal cases with *E.coli* O157:H7 infection was occurred in age from (first month - 5years) at percentage (5.45%) while the percentage (3.07%) appeared in age (6-12) years. *E.coli* O157:H7 in children with Urinary Tract Infection showed a percentage (2.4%) in children with age from (1 month- 5) years.

In conclusion this, study revealed the importance of *Escherichia coli* O157:H7 in infection children from birth to ten years and causing a severe intestinal and urinary tract infection.

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Introduction

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world. This was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea traced to consumption of hamburgers at common chains of fast food restaurants (Riley et al., (1983). *Escherichia coli* O157:H7 causes sporadic outbreaks of intestinal disease in man. It has achieved notoriety as the cause of a number of large food- and water-borne outbreaks (Ogden et al., 2002). Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is an emerging pathogen that causes acute human gastroenteritis and hemorrhagic colitis (Rabinovitz et al., 2012) and A major causative agent of severe UTI in children (Navidinia et al 2012).

Some antibiotics may cause *E.coli* lysis and liberate the free Shiga toxins in the intestinal tract (Wong et al 2000), and enhance the expression of Shiga toxins genes (Zhang et al 2000), the antimicrobial treatment is contraindicated for human *E.coli* O157:H7 infections. However, such treatments may be recommended for cystitis and

pyelonephritis other than hemorrhagic colitis all caused by *E.coli* O157:H7 (Griffin, 1995). The aim of this study was to study the prevalence of *E.coli* O157:H7 in intestinal and Urinary tract infection in children in pediatric hospital.

Material and Methods

Children with different ages between (1 month- 10 years) from both sex male and female in Pediatric Hospital of Karballa governorate:

- Two hundred twenty five stool samples were collected from children suffering from diarrhea.
- Two hundred children had complication related to (UTI) urinary tract.
- **Bacterial Isolation:** Each sample was cultured initially on MacConky agar and Eosin methylene blue agar then incubated aerobically at 37 C° for 24 hours. Staining of single colony from the growth by gram stain, then Biochemical tests (Kligler Iron medium (KI), Ureas test, Indole test, Motility, Citrate utilization test) were done on isolated bacteria to confirm diagnosis (Quinn et al., 2004)

Confirmation of *E. coli* O157:H7:-

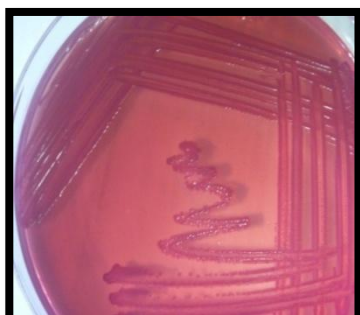
1. Culturing on Sorbitol MacConkey agar plus cifaxime potassium tellurite (SMA-CT)
2. Culturing on Chrom agar™ *E. coli* O157:H7
3. Potassium Cyanide (KCN) Test.

Serological Test :

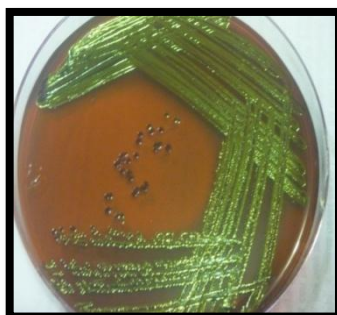
(Latex agglutination Test for *E.coli* O157:H7) serotyped by Latex agglutination test. kit (Wellcolex *E.coli* O157:H7, Remel).

Results & Discussion

Results of *E.coli* Isolation: The results of culturing samples showed different morphological characteristics of *E.coli* colonies on different media, On MacConkey agar the colonies appeared red /pink color, on Eosin Methylene Blue the colonies appeared as metallic sheen (Figure 1,2). Isolated bacteria appeared as gram negative rod, non spore forming under light microscopic suspected as *E.coli* (Figure 3).



Figure(1)*E.coli* on MacConkey agar



Figure(2)*E.coli* on Eosin Methylene Blue agar



Figure (3) : The shape of *E.coli* .stained by gram stain

The red /pink color on MacConkey agar occurred due to utilizing the lactose available in the medium with surrounding areas of precipitated bile salts, while Eosin Methylene Blue (EMB) agar which used for selection and isolation purposes, and considered as a rapid and accurate method of distinguishing *E. coli* from other gram-negative pathogens. the green metallic sheen color indicate the vigorous fermentation of lactose and acid production which precipitates the green metallic pigment (Quinn *et al* .,2004).

Biochemical identification: The biochemical tests of the isolates bacteria gave different results as showed in table (1).

Table (1): The results of some biochemical tests of *Ecoli* spp.

Biochemical test	Result
Oxidase test	–
simmon citrate test	–
Urease test	–

Kligler Iron test	Yellow/Yellow with gas production
Indol test	+
Motility test	+

To aid in the more definitive identification of *E.coli*, there is a series of biochemical tests that can be used to differentiate which is closely related with its metabolism. These tests were designed to identify various metabolic properties of this bacteria like Oxidase test with a violet or purple color change that indicated of *E.coli* with Cytochrome oxidase enzyme, while urease is negative due to absence of urease enzyme lead to unhydrolyzed urea, and simon citrate give negative result because *E.coli* cannot use citrate as sole of carbone source, and it can motile in motility media, *E.coli* produce indole from tryptophan by Indole test and a result appear as rose ring in upper test tub and it have ability to fermentation lactose and glucose in Kligler test, (Quinn et al 2004).

Confirmation of *E. coli* O157:H7.

1.Culturing on Sorbitol MacConky agar plus cefixime potassium tellurite (SMA-CT).

The suspected colonies of EHEC appeared small, circular and colorless with smoky center (1-2) mm in diameter on SMA-CT Figure (4)

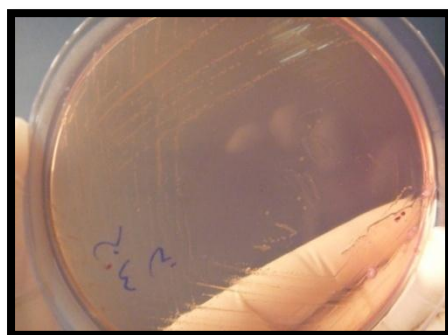


Figure (4) colonies of *E.coli* O157 H7 on SMA-CT.

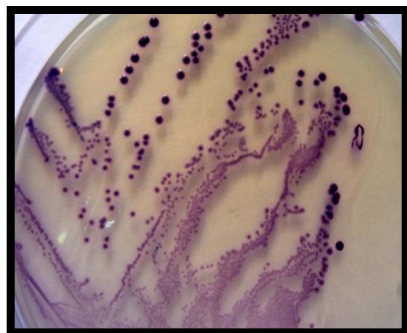
The result of growth on SMA-CT was similar to many studies, March and Ratnam (1989) reported that sorbitol MacConkey agar was useful in screening for *E.coli* O157: H7 in fecal specimens and Doyle, (1991) found Sorbitol MacConkey agar is a variant of traditional MacConkey agar used in the detection of *E. coli* O157:H7, that differs from most other strains of *E. coli* in being unable to ferment Sorbitol. In Sorbitol MacConkey agar, lactose is replaced by Sorbitol. Most strains of *E. coli* ferment Sorbitol to produce acid but *E. coli* O157:H7 cannot ferment Sorbitol, this method exploits the fact that *E. coli* O157:H7 unlike 90% of *E. coli* isolates does not ferment Sorbitol rapidly. aylor et al., (2002); Bielaszewska et al., (2005); Orth et al., (2007) showed the resistance of *E.coli* O157:H7 for tellurite and therefore grows in concentration often that inhibit most other *E. coli*, This characteristic together with its inability to ferment Sorbitol has been exploited in selective strategies to isolate EHEC O157:H7 from feces, food and the environment.

2. Specific Biochemical Test.

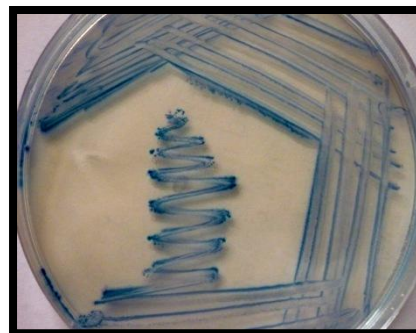
Culturing of *E.coli* on potassium cyanide (KCN) broth didn't showed any turbidity, this test was confirmed test to *E. coli* especially *E.coli* O157:H7, this result was compatible with Strockbine et al., (1998) who mentioned that *Escherichia hermannii* is biochemically similar to *E. coli* O157:H7, therefore the main biochemical test that required to distinguish between them was their ability to grow in the presence of potassium cyanide (KCN). *E. coli* O157:H7 was unable to grow in the presence of potassium cyanide while *E. hermannii* is able to grow in potassium cyanide broth. Our test was done after culturing on sorbitol macConky media, also each Al-Charrakh and Muhana,(2010); Ahmed et al.,(2011) and Bassam et al., (2012) whom founded that the confirmation of *E. coli* O157: H7 by culturing on potassium cyanide (KCN) broth.

3- Culturing on Chrom agar™ *E. coli* O157: H7:

The culturing of isolated *E.coli* on CHROM agar showed different color colonies like *Ecoli* O157:H7 showed mauve color (figure, 5), while other species of *E.coli* showed blue color colony (Figure, 6).



Figure(5) *E. coli* O157:H7



Figure(6) other *E. coli* species

Our results showed the importance of Chrom agar media in diagnosis of *E. coli* O157:H7. A similar results reported by other researchers. Bettelheim, (1998) found that *E. coli* O157:H7 utilizes one of chromogenic substrates which produce mauve colored colonies. The growth of mauve colored colonies is considered presumptive identification for *E. coli* O157:H7 on Chrom agar™ O157. Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue green colored colonies or, if none of the chromogenic substrates are utilized, colonies may appear as a natural color. Tarr et al .,2005 ; Philips et al .,(2005) showed that The improved diagnostic performance and efficiency of Chrom agar would allow more appropriate management of *E. coli* O157 cases and outbreaks. A similar study of Tavakoli et al., (2008) showed that chromogenic media have more advantage and can be an appropriate alternative for conventional and routine procedure.

Research conducted all over the world has been shown that these technique have higher specifically, exculsivity and performance in compare with other technique and indicated that Chrom agar is an effective supplemental medium for the isolation of probable STEC strains (Ngwa et al ., 2013).

4-Serotyping test (Wellcolex *E. coli* O157:H7, Remel)

Figure (7) demonstrated the positive and negative result according agglutination which appeared on card test, the non-sorbitol fermenting cultures colonies from sorbitol MacConkey agar plus cifaxime potassium tellurite (SMA-CT) were tested for identification of both O157 & H7 antigens. Isolates that gave a positive reaction for the O157 antigen were sub-cultured overnight on blood agar for the detection of H7 flagellar antigen because some of the *E. coli* O157 strains are non motile. *E. coli* O157:H7 detected by using this test showed that red color agglutination indicated a positive result for (O antigen) in comparison to clear red color of the control and the blue color agglutination indicated positive result for (H antigen) in comparison to clear blue color of the control . According to this results of latex agglutination test illustrated in figure (7) revealed that (3) urin samples and 11 stool samples gave positive results to *E. coli* O157:H7.

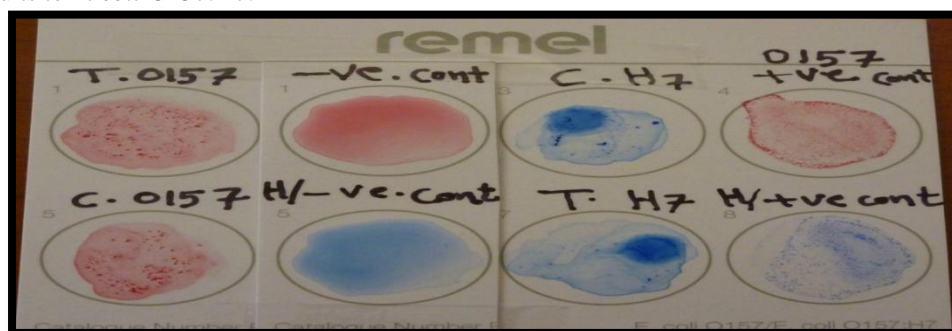


Fig. (7) : Serotyping of *E. coli* O157 :H7 by Wella colex color *E. coli* O157:H7 test.

In present study latex agglutination test considered as a rapid detection method to reduce the time also to eliminate other serotypes of pathogenic *E. coli* which have the cultural and biochemical character, this result agree with March and Ratnam (1989) who evaluated latex test as a rapid presumptive detection of *E. coli* serotype O157:H7 when determined by laboratory trials. A similar finding mentioned by Komatsu et al., (1997) who reported that The rapid detection method of *Escherichia coli* O157 in feces by using the latex agglutination test also Karmali et al ., (1999) showed that the latex agglutination method is highly sensitive and specific for the detection and

characterization of VTs in culture filtrates of human *E. coli* isolates. The test is rapid, reliable, and easy to perform; its results are easy to interpret; and it should allow testing for VT to become more widely performed.

5- Prevalence of infection with *E. coli* O157:H7 in diarrheal and UTI Children:

Microbiological examination of stool samples that collected from children revealed that 126 out of 230 were positive to *E. coli*. From 126 *E. coli* only 11 isolates were positive to *E. coli* O157:H7 at a percentage (4.78%). The number of *E. coli* isolates in urine samples were 98 from 200 samples, only 3 isolates gave positive results to *E. coli* O157:H7 at a percentage (1.5%) (Table, 2; Figure,8) .

Table (2):Number of *E. coli* O157:H7 isolated from urine and stool of children.

Children samples	Number of samples	Total Number (%) of <i>E. coli</i>	No. & (%) of <i>E. coli</i> 157: H7 isolates
Stool	230	126 (54.7%)	11 (4.78%)
Urin	200	98 (49%)	3 (1.5%)
Total	430	224 (52.09%)	14 (6.25%)

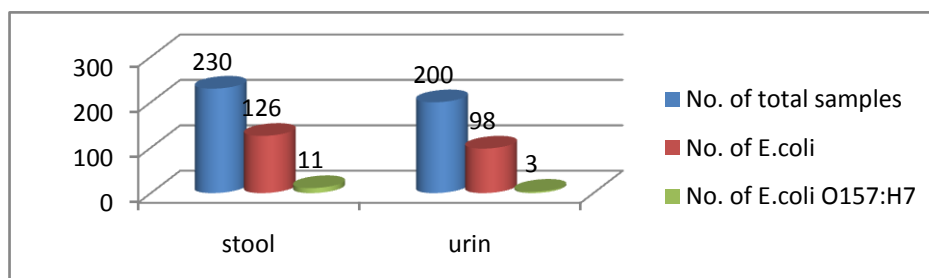


Figure (8) Number of isolated *E. coli* & *E. coli* O157:H7 in stool and urin samples

The result in present study of isolation at (4.78%) from stool samples is in agreement with Máttar *et al.* ,(1997) who showed that *E. coli* O157:H7 with a prevalence rate 4.7% in children with acute gastroenteritis, and Shebib, (2000) reported non sorbitol forming isolates rate at 5% and 1.14% respectively, *E. coli* isolates were serotyped as *E. coli* O157:H7 with (5.7%) isolated from stool in diarrheal patients in Basrah city by Mohmmed *et al.* ,(2011), Other Iraqi studies such as Al- awwadi *et al.* ,(2012) also diagnosis *E. coli* O157:H7 serotyped as a cause of diarrhea in children with ratio 4% with (2 bloody samples) from the total samples where as the *E. coli* O157:H7 strains can cause hemorrhagic colitis in humans. A study conducted by Elaine *et al.* ,(2013) who recorded that proportion of *E. coli* O157:H7 isolations bacterium from diarrheal cases of children in USA (3%). Vally *et al.* ,(2012) reported the lower percentage of infection with *E. coli* O157:H7 in children.

Urine samples data demonstrated that three *E. coli* O157:H7 were isolated (1.5%). This result compatible with result of Navidinia, *et al.*,(2012) who showed that (2.3 %) of *E. coli* isolated from children's urine with UTI was Enterohemorrhagic. While high rate reported by Craig *et al.* ,(2000) who found among the 71 children, 9 (13%) received antibiotics and the hemolytic-uremic syndrome developed in (14%) also Wong *et al* (2012) showed that 36 (14%) out of 259 children developed with HUS and demonstrated that children who received antibiotics during the diarrhea phase more frequently developed HUS than those who did not received antibiotic. Mody *et al.* ,(2012) found that (70.7%) of infection with HUS-causing agents: STEC O157 and mentioned that early stool collection for *E. coli* O157 culture and Shiga toxin testing of all children with possible bacterial enteric infection will increase detection of STEC strains causing HUS,.

The present study is in higher than with other studies with Hemorrhagic Uremic Syndrom such as the study of Ong , *et al* (2012) ; Gouali and Weill ,(2013) they showed that *E. coli* O157:H7 in a low percentage.

During our study we observed Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the vast majority of urinary tract infections (UTIs) when isolated from children's urine samples at (49%). So *E. coli* is the most frequent pathogen inducing urinary tract infection (UTI). this result also supported by Bouallegue *et.al.*, (2004) in Tunisia

who reported a high rate (71%) of *E.coli* infection from urine samples. Kasper, *et al.*, (2005) found that *Escherichia coli* (*E. coli*) accounts for (80%) of UTIs, Friedman *et al.*, (2006) observed that (76.9%) UTI in children and infants caused by *E coli*. also Nicolle, (2008) mentioned that *E.coli* is the most common organism implicated in UTIs (80–85%) and Zahera, *et al.*, (2011) reported *E.coli* isolates at (30%).

5-Prevalence of *Ecoli* O157:H7 in intestinal and UTI Children according to age:

According to age effect, the study revealed that most of the diarrheal cases were occurred from first month to 5years at percentage (5.45%) of all *E.coli* O157:H7 isolates, while the percentage (3.07%) appeared in age (6-12) years (Table 2). *E.coli* O157:H7 in children with Urinary Tract Infection showed a percentage (1.5%) in children with age (first month - 5 years) but in age more than five years there is no evidence of isolation (table, 2).

Table (2): Distribution of *Ecoli* O157:H7 serotypes according to age in children.

Age	No. of Children infected with diarrhea	No. of <i>E.coli</i> O157:H7 isolates in fecal samples of diarrheal children.	No. of Children infected with UTI	Number of <i>E.coli</i> O157:H7 Isolates In urin samples of UTI childern.
First month– 5 year	165	9(5.45%)	125	3(2.4%)
6year– 12year	65	2(3.07%)	75	0
Total	230	11(4.78%)	200	3(1.5%)

In the present study the results enrolled according to age demonstrated that children less than five years more susceptible to infection, this criterion is consistent with many researchers, Slutsker *et al*; (1997) found that the highest age-specific isolation proportions from fecal specimens for *E. coli* O157:H7 were in patients at 5 to 9 years of age (0.90%) and 50 to 59 years of age (0.89%).

Delignette (2008) showed that children less than 5 years of age are roughly 5 times more susceptible to the pathogen than children more than 5 years. While Giacometti *et al.*, 2012 studied the risk of illness with *E.coli* O157:H7 linked to raw milk consumption increased in children under five years, found the highest proportion (0.05 %) in compared with children between (5-12) years (0.5%). In addition, this result is compatible with previous studies of *E.coli* O157:H7 infection in Iraq by Tawfeek_ *et al* (2002) who mentioned that the peak incidence was at 3-6 months at retrospective study of all infants and children admitted to two pediatric teaching hospitals in Baghdad city complaining of acute diarrhea.

Our results showed high prevalence in children under less than years belong to their immune systems are not fully developed, and may due to the absence of breast feeding and using bottle only (artificial milk) might especially increase the probability of the infection among children less than 2 years age. Sucking their thumbs, dropped dummies or toys can increase risks to infants and toddlers, who also need supervised hand washing. and may the infection is higher among infants than among older children and adults because infants with diarrhea are more likely to be brought to medical care and have stool cultured.

Our results similar to results of Paton and Paton, (1998) they record approximately 5–10 percent of individuals with hemorrhagic colitis progress to HUS, especially for children less than five years of age. Boldtsetseg *et al.*, (2005) suggested that prolonged diarrhea (>3 days) in 3 years of age may increase the risk of HUS. Rivas, *et al* (2008) found the highest rates of HUS globally in Argentina children aged <5 years. Loirat (2013) indicated that *Escherichia coli* (STEC) infection, mostly the O157:H7 serotype. STEC-HUS is less frequent in adults, Half of patients require dialysis at the acute phase, and elucidated that the majority will recover normal renal function but approximately 30% will suffer renal sequelae.

EHEC are a cause of different troubles ranging from mild diarrhea to haemorrhagic colitis which might be complicated by HUS in young children and thrombocytopenic thrombotic purpura in adults (Gouali and Weill,2013). Our result suggest that Urinary tract infections (UTI) caused by enterohemorrhagic *Escherichia coli* (EHEC) especially in infants and children less than five years because most UTIs result from fecal-perineal-urethral retrograde ascent of uropathogens, fecal and perineal flora are important factors in the development of a UTI, In addition early identification is very essentially because if uncorrected, they may serve as a reservoir for bacterial persistence and result in recurrent UTI.

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