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Isolation and characterization of *E. coli O157:H7* from human and animals

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Abstract

This study was conducted to show the importance of *Escherichia coli O157:H7* as an important zoonotic pathogen, which has ability to transmit from animals to human in Iraq. One hundred fifty fecal samples (50 stool samples from diarrheal children and 50 from cows and 50 from sheep) were collected for isolation of *E. coli O157: H7*. All samples were cultured on MacConkey and Eosin Methylene blue agar. Each *E. coli* growth was confirmed by Gram stain and biochemical tests. Then, all isolated *E. coli* were sub cultured on Sorbitol MacConkey agar plus cifixime potassium tellurite (SMA-CT). The diagnosis was confirmed by Chrom agar™ *E. coli O157:H7* and incubated aerobically at 37 C° for 24 hours. Latex agglutination test was used to all isolates of *E. coli O157: H7* to confirm the serotype. The results showed that *E. coli* were isolated in 40 out of 50 diarrheal children stool samples, where only 2(4%) from these isolates were confirmed as *E. coli O157:H7*. The number of *E. coli* isolates from cows and sheep samples were 48 out of 50 and 45 out of 50 respectively, where only 12 (24%) and 10 (20%) isolates were *E. coli O157:H7* respectively. The study revealed that most diarrheal cases with positive *E. coli O157:H7* were detected in two children aged 1year and 4 years. In conclusion, this study revealed the importance of human and ruminants to act as a reservoir for *Escherichia coli O157:H7*.

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Introduction

Escherichia coli O157:H7, was first identified as a human pathogen in 1982 (Riley *et al.*, 1983). Enterohemorrhagic *Escherichia coli (EHEC) O157:H7* is an emerging pathogen that causes acute human gastroenteritis and hemorrhagic colitis (Rabinovitz *et al.*, 2012). Enteropathogenic *Escherichia coli (EPEC)* is the most

important cause of persistent diarrhea in children, particularly in developing countries. Animals serve as reservoirs for pathogenic *E. coli*, and compelling evidence for cross-species *EPEC* transmission exists (Swennes *et al.,* 2012). The main reservoir for this group is bovine bowels, and infection mainly occurs after ingestion of contaminated water and food **(**Regua-Mangia *et al.,* 2012**).** Cattle are the principal reservoir of *E. coli O157:H7* and the rumen has been shown to harbor this pathogen. It is found in the distal portion of the bovine gastrointestinal (GI) tract, with the recto-anal junction identified as the predominant colonization site (Naylor *et al.,* 2003; Arthur *et al.,* 2010). Once colonized, an animal can shed *E. coli O157:H7* organisms in the feces. These animals have been referred to as super-shedders and are reported to be responsible for increased transmission of *E. coli O157:H7* within cattle production environments, as well as having effects on contamination of the cattle hide and carcass (Chase-Topping *et al.,* 2008). The *STEC* has also been isolated from the feces of other farm animals, including other ruminants such as sheep, and water buffalo, and non-ruminants (horses, dogs, rabbits, and pigs) (Ørskov *et al.,* 1987; Caprioli *et al.,* 2005). *Escherichia coli O157:H7* has been found in cattle faces from most areas of North America, Canada, United Kingdom, Scotland, Netherlands, Finland, Italy, Japan, France, South America, and Australia suggesting that this organism is ubiquitously present on cattle farms. There are however, other regions, such as Scandinavia, Africa, and Norway that report a very low prevalence of *E. coli O157:H7* (Johnsen *et al.,* 2001; Vold *et al.,* 1998). The aim of this study was to isolate and characterize *E. coli O157:H7,* in addition to investigate the rate of infection in children and animals.

Materials and Methods

Samples

- Fifty stool samples were collected from children suffering from diarrhea of both genera less than 10 years of old from Pediatric Hospital.
- One hundred fecal samples (50 from cows and 50 from sheep) were collected from field's animal.

Isolation and Characterization of *E. coli O157:H7*

Culturing and Biochemical tests were done according to (Quinn *et al.,* 2004). All fecal samples were cultured on MacConkey and eosin methylene agar and incubated at 37 C° for 24 hours (Al- awwadi *et al.,* 2012). The Kligler Iron medium (KI), Ureas test, Indole test, Motility and Citrate utilization were the biochemical tests, which have been done on all suspected colonies. The confirmation of *E. coli O157:H7* was done by culturing of suspected colonies on Sorbitol MacConkey agar plus cifixime potassium tellurite (SMA-CT), then culturing on Chrom agar™ *E . coli O157:H7*. The serotyping of isolated bacteria was confirmed by *E. coli O157* Latex agglutination test (Kit from Wellcolex *E. coli O157:H7*, Remel).

Results and Discussion

E. coli isolation

Different morphological shape and color of *E. coli* colonies were appeared on different media. The colonies revealed red /pink color On MacConkey agar and metallic sheen on Eosin Methylene Blue. The Gram stain of suspected *E. coli* colonies revealed, negative non-spore forming rod. The isolated bacteria gave different reaction in biochemical tests. It gave negative for oxidase, simmon citrate, Urease tests and positive for indole and motility tests. The Kligler Iron test showed Yellow/Yellow with gas production. The suspected colonies of EHEC appeared small, circular and colorless with smoky center (1-2) mm in diameter on SMA-CT. On Chrom agar the colonies of *E. coli O157:H7* showed mauve color. *Escherichia coli O157:H7* are commonly identified by culturing on different media and sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC), this result was compatible with (Chapman *et al.,* 1991). Our results showed that Chrom agar aids in diagnosis of *E. coli 0157:H7*. *E. coli O157:H7* utilizes one of chromogenic substrates and produce mauve colored colonies. While non- *E. coli O157:H7* organism may utilize chromogenic substrates resulting in blue to blue green colored colonies. These observations are in agreement with Bettelheim, (1998); Tarr *et al.,* (2005); Philips *et al.,* (2005) and Tavakoli *et al.,* (2008). They recorded that the using of Chromogenic media have more advantage and can be an appropriate alternative for conventional and routine procedure. Ngwa *et al.,* (2013) also found that the Chrom agar is an effective supplemental medium for the isolation of probable STEC strains.

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

Escherichia coli colonies from (SMA-CT) were tested for identification of both *O157:H7* antigens by Wellcolex *E. coli O157:H7*, Remel. The isolates that gave a positive reaction for the *O157* antigen were sub-cultured overnight on blood agar for the detection of flagellar antigen (*H7*). 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. Results agree with March and Ratnam, (1989), who evaluated latex test as a rapid test for diagnosis of *E. coli* serotype *O157:H7*. However, Karmali *et al.,* (1999) showed that this test method is highly sensitive and specific for the diagnosis of *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.

Prevalence of infection with *E. coli O157:H7* in diarrheal Children

Stool samples that collected from diarrheal children revealed that 40 out of 50 were positive to *E. coli*. There were only 2 isolates from this 40 *E. coli* were positive to *E. coli* O157:H7 at a percentage (4 %) (Table1).

Table (1): Rate of infection of *E. coli O157:H7* in children

Origin of samples	No. of samples	Total No. of *E.coli*	No. of *E.coli O157:H7*	%
Children stool	50	40	2	4%

These results showed that the rate of infection in stool samples were 4%, which is in agreement with Máttar *et al.,* (1997). They recorded that the prevalence of *E. coli O157:H7* was 4.7% in children with acute gastroenteritis. In Basra/ Iraq, *E. coli O157:H7* was isolated by Khudor *et al.,* (2011) at a percentage (5.7%) from stool in diarrheal patients. Previous studies in Iraq were compatible with the results of the present study. Also, Basil *et al.,* (2012) isolated Verotoxigenic *E. coli O157:H7* from Raw milk Using Duplex PCR, and Al- awwadi *et al.,* (2012) reported *E. coli O157:H7* serotype as a causative agent of bloody diarrhea in children at the same isolation percentage 4%. Another study in Iraq (Al –Dawmy and Yousif, 2013) revealed the importance of *Escherichia coli O157:H7* as a children pathogen and a causative of a severe intestinal and urinary tract infection from birth to ten years. However, other studies recorded lower percentage of infection with *E. coli O157:H7* in children (Vally *et al.,* 2012). In addition, Elaine *et al.,* (2013) recorded that proportion of *E. coli O157:H7* from diarrheal cases of children in USA. In Iran, Aslani *et al.,* (2003); Salmanzadeh-Ahrabi *et al.,* (2005) and Jomezadeh *et al.,* (2009) recorded that *E. coli O157:H7* is a common cause of bloody diarrhoea in developed countries, but its incidence in developing countries including Iran is not clear. The limited prevalence data in foods and animals has made the assessment of risks difficult, also the options for management and control are unclear. A few studies have reported the isolation and characterization of *STEC* in humans in Iran. The number of *E. coli* isolates in cows fecal samples were 48 out of 50 samples and only 12 isolates gave positive results to *E. coli O157:H7* at a percentage (24%). However, sheep fecal samples revealed 45 *E. coli* isolates out of 50 fecal samples and10 isolates characterized as *E. coli O157:H7* (Table 2).

Table (2) Rate of infection of *E. coli O157:H7* in animals

Origin of samples	No. of samples	Total No. of *E.coli*	No. of *E.coli O157:H7*	%
Cows fecal samples	50	48	12	24%
Sheep fecal samples	50	45	10	20%

The results of *E. coli O157:H7* isolation from cows and sheep was similar to other researcher, Matthews *et al.,* (2006). They presented data suggesting that 20% of the *E. coli O157:H7* infections in cattle on Scottish farms were responsible for 80% of the transmission of the organism between animals. Another study showed that 9% of the animals shedding *E. coli O157:H7* at harvest produced over 96% of the total *E. coli O157:H7* fecal load for the group (Omisakin *et al.,* 2003). Arthur *et al.,* (2009) have shown that 95% of feedlot pens containing at least one super shedder had *E. coli O157* prevalence rates on cattle hides exceeding 80%. The results of Keen

and Elder (2002) suggested that viable *STEC O157* may be isolated from the oral cavity, hide surfaces, and feces of a high percentage of fed beef cattle and that bacterial culture of feces alone generally underestimates the percentage of fed beef cattle from which *STEC O157* can be isolated. Al-Charrakh and Al-Muhana, (2010) found that dairy cattle had been implicated as principal reservoir of Verotoxin-Producing *E. coli (VTEC),* with undercooked ground beef and raw milk being the major vehicles of food borne outbreaks. *VTEC* had been implicated as an etiological agent of individual cases and outbreaks in developed countries. The epidemiology of *E. coli O157:H7* in sheep is similar to that of cattle. It is including the transient faecal excretion in individual sheep, with a higher prevalence detected in the summer, which is ranging from 31% positive in June (Kudva *et al.,* 1996). The prevalence levels for individual sheep in the United Kingdom was ranged from 2.2 to 7.4% (Chapman *et al.,* 2001). The study also showed that sheep may shed at the same time more than one strain of *E. coli O157:H7*. In addition, the excretion of *E. coli O157:H7* does not prevent re-colonization of sheep with *E. coli O157:H7* (Kudva *et al.,* 1997).

In conclusion, this study showed that cattle and sheep were acted as the main natural reservoir for *E. coli O157:H7* and able to transmit the disease to human.

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