Detection of *E. coli* O157: H7 bacteria in Iraqi soft cheese in some areas of Baghdad

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Abstract:

in this study 25 samples of Iraqi soft cheese were collected from different areas in Baghdad. The total number of isolates was 82, the number of fermented isolates of sorbitol was 60 and unfermented isolates of sorbitol was 22.

The diagnosis of non-fermented isolates of sorbitol was carried by cultural, Morphological and Biochemical diagnosis, the results of the biochemical tests initially showed that the isolate belongs to the *E. coli* O157: H7 serotype.

The latex test results indicate a clear appearance of granularity (agglutination) when using drops of O157 Test Latex reagent compared to control treatment.

Key words: *E. coli* O157: H7, Iraqi soft cheese

Introduction:

Escherichia coli is a species that normally exist in the intestines of people, animals and found in the environment and foods, was first described by Theodor Escherich in 1885. More than 700 serotypes of *E. coli* have been known. Most of them are harmless, but a few strains enteropathogenic, cause severe abdominal cramps, bloody diarrhea and vomiting (Rahal *et al*., 2012).

in 1982, a new bacterial pathogen, *Escherichia coli* serotype O157:H7 was recognized asa human pathogen, because two outbreaks involving contaminated hamburgers, *E. coli* O157: H7 is not fermenting sorbitol and non-producing β-glucuronidase enzyme, (Schutz, *et al.*,2017)

Escherichia coli O157:H7 produces verocytotoxin, also known as Shiga-like toxins, these toxins are heat stable and closely related to the potent cytotoxins produced by *Shigella dysenteriae* type 1 and *has* become an important food and waterborne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome, Erickson, *et al*. (2019). The bacteria produce two Shiga toxins that differ in immunologically, biological, physiochemically and antigenically effects (Celsa,2014).

E. coli O157: H7 Transmission through consumption of contaminated foods, such as raw or undercooked meat and meat products and raw milk,

transmitted by person-to-person and from animal to person.,. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (Atnafie, *et al*.2017, Kiranmayi, *et al*.2010). Small doses of (10 -100) cells *Escherichia coli* O157:H7 may lead to infection (Lahmer, *et al.* 2017).

The aim of the current study is detecting *E. coli* O157 H7 in Iraqi soft cheese in different areas of Baghdad.

Materials and methods

Samples: Twenty-five samples of Iraqi soft cheese were collected from different areas in Baghdad, including Sadr city, Al-Bayaa, Al-Shula, Al-Hurriya, and Abu Ghraib, with 5 samples for each region. Samples were collected in clean, sterile cans.

After the initial activation process using Trypticase Soya Broth medium (TSB) and incubation at 41.5 ° C for 24 hours, the isolation process was performed using the dilutions prepared from the previously activated samples by the pour-plate technique on Cefixime-Tellurite-Sorbitol MacConkey Agar (CT-SMAC)(Sheikh, *et al*.2013).

The pale brown colonies were transferred by streak plate method over the same medium until pure colonies were obtained. The pure isolates were preserved on slants of nutrient agar in the refrigerator for daily use and some were preserved by freezing after adding glycerol.

The diagnosis of the colonies was performed on the selected medium (SMA), which was incubated at 37 ° C for 24 hours, and recorded the shape, surface, edges and height of the colonies.

Indole test: This test was used to know the ability of bacteria to produce indole from tryptophan. The medium of peptone water was prepared according to the manufacturer's instructions and distributed on test tubes (5 ml per tube). The tubes were sterilized with an autoclave and then left to cool down. Inoculation with peptone water and the tubes were incubated at 37 ° C for 24 h. After the end of the incubation period, add 5 drops of Kovac's reagent to each tube and shake calmly, as the appearance of a red ring is evidence of the positivity of the test.(Welde, *et al*.2020).

Methyl red test: This test was used to find out the ability of isolates to ferment glucose and produce acids. Inoculation of MR-VP medium and incubated at 37 ° C for 24 hours, then 3-4 drops of red methyl reagent were added to the tubes and shaken gently. Changing the color of the medium to red is indicative of a positive test.(Welde, *et al*.2020)).

Voges-Proskauer test: This test is used to find out the ability of bacteria to ferment glucose and produce acetone. Inoculate the MR-VP medium and incubated at 37 for 24 hours, then added Voges-Proskauer reagent and shaken gently for 30 seconds. A change in the color of the medium to red within 2-5 minutes is an indication that the test is positive (Welde, *et al*.2020).

Simmon's citrate test: This test is used to find out the ability of bacteria to consume sodium citrate as the sole source of carbon and ammonium salts as a source of nitrogen. Simmon's citrate medium was prepared according to the manufacturer's instructions, inoculate the medium and incubate at 37 ° C for 48 hours. The appearance of a bacterial growth with a change in the color of the medium from green to blue is considered a positive test..(Welde, *et al*.2020).

Oxidase test: This test was used to find out the ability of bacteria to produce oxidase and to differentiate the intestinal strains. One drop of the oxidase reagent was added to a single colony growing on the nutrient agar medium, and the colony's coloration to violet within 5-10 seconds is evidence of the positive test (Benson ,2002).

Catalase test: Trypticase soy broth medium was inoculated and incubated for 24 h at 37 ° C, then drops of hydrogen peroxide (3%) were added. The appearance of bubbles is considered a positive test ,(Thenmozhi,2010).

Motility test: The test was performed using SIM (Sulfide, Indole, Motility) medium. The medium was inoculated with non- sorbitol fermented isolates using a sterile needle and incubated at 37 ° C for 24 hours. The spread of the growth away from the stab line, forming a tree-like shape, indicates that the bacteria are mobile, but if the growth in the stab line only indicates that the bacteria are not mobile ,(Thenmozhi,2010).

Latex agglutination test:It is one of the important confirmatory tests for the diagnosis of *E. coli* O157: H7. The test was performed by transferring a swab of the bacterial growth and mixing it with distilled water, then adding drops of O157 test latex. The appearance of granulation is an indication that the isolate possesses the O157 antigen and the absence of granulation indicates that the isolate does not possess this antigen.(Medina,et al.2012).

Results and discussion

Detection of E. coli O157: H7 in cheese samples:

The process of detecting the presence of *E.coli* O157: H7 bacteria in cheese samples, which were collected from different areas of the city of Baghdad, was carried out through the isolation process, which is the main step in detecting the presence of this bacteria or not in the samples under study.

25 samples of Iraqi soft cheese were collected from different areas including Sadr City, Al-Bayaa, Al-Shula, Al-Hurriya and Abu Ghraib, with 5 samples for each region

Isolation was performed using Cefixime-Tellurite-Sorbitol MacConkey Agar (CT-SMAC) medium which is a selective medium and limits the growth of some non-fermented species of sorbitol that may be present in the isolation model with *E. coli* O157: H7.

Table (1) shows the total number of 72 isolates obtained for different cheese samples.

The total percentage of fermented isolates of sorbitol was 83.33% (Figure 1A), while the non-fermented isolates of sorbitol were 16.67% (Figure 1B), which could include the bacteria *E. coli* O157: H7, as it is characterized by its inability to ferment sorbitol compared to the other species of *E. coli*, It appeared in a pale golden color while the fermented sorbitol was purple (Figure 1A).

A **B**

Figure (1): A: Pure, Fermented Colonies of Sorbitol and B: Pure, Unfermented Colonies of Sorbitol

The presence of these large numbers of fermented and non-fermented of sorbitol may be due to several reasons such as the poor quality of the raw material used in the manufacturing process, its high content of microorganisms, the inefficiency of the thermal treatments used during the manufacturing process and the unhealthy procedures used in the preservation and handling process.

Al-Gamal *et al* (2019) andHamzah,*et al*. (2013) used Cefixime-Tellurite-Sorbitol MacConkey Agar (CT-SMAC) in the isolation of*E.coli* O157: H7 from different food sources, while Dadi, *et al*. (2020) used Sorbitol MacConkey Agar.

Table (1) Total number of isolates, number of isolates fermented of sorbitol (purple color) and non-fermented sorbitol (pale golden color) and their isolation regions.

Isolation areas	The total number of isolates	Number of fermented isolates of Sorbitol	Number of unfermented isolates of Sorbitol
Sadr City	**15**	**10**	**5**
Al-Bayaa	**12**	**11**	**1**
Al-Shula	**14**	**12**	**2**
Al-Hurriya	**15**	**13**	**2**
Abu Ghraib	**16**	**14**	**2**
Total	**72**	**60**	**12**

The differences in the prevalence of *E. coli*O157: H7 may be due to the source of samples, method of sampling, environmental conditions, and health and cultural awareness of the producers and vendors.

Diagnosis of non-fermented isolates of sorbitol

Cultural diagnosis: The initial diagnosis of the non-fermented sorbitol isolates based on their culture characteristics was performed on CT-SMA after incubation for 24 hours at 37 ° C. The colonies appeared pale golden to colorless because they did not ferment sorbitol, the surface of the colonies was smooth, convex and sticky, and the edges of the colonies were whole (Table 2).

Morphological diagnosis: The morphological characteristics of non-fermented sorbitol isolates were studied by staining them with Gram staining and then examining with Compound Optical Microscope(1000X) in order to determine their morphological properties.

The results showed that all the isolates were distinguished by their short straight rods shape, with single or bilateral groupings, and were negative for cream pigment, pinkish-reddish color (Fig. 2) and Table (2), and these characteristics are in agreement with those mentioned inPark,*et al*. (2011) and Shinde ,*et al*.(2020).

Table (2): Cultural and morphological characteristics of non-fermented sorbitol isolates.

Culturalcharacteristics	
Colony shape	**Circular**
Colony color	**Pale golden or colorless**
Margin	**Even**
Elevation	**Convex**
Surface of colony	**Smooth (sticky)**
Morphological characteristics	
The shape of the cells	**Short rod**
Gram's stain	**Negative**

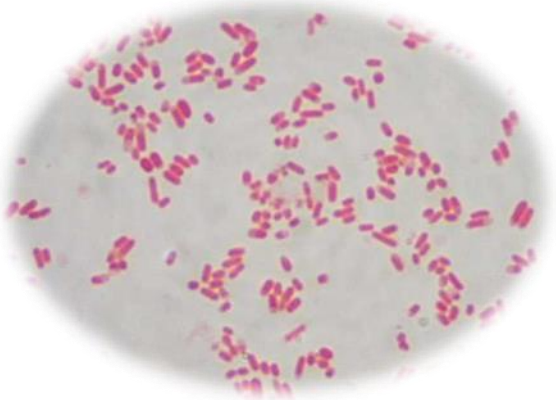


Figure (2): Non-fermented bacterial isolates of sorbitol using a compound light microscope (1000x)

Biochemical tests: Table (3) shows the results of biochemical tests for the diagnosis of non-fermented sorbitol isolates. The positive of indole test for only one isolate of fermented sorbitol isolates is shown by the change of the pale-yellow color to the red color due to the interaction of the indole with the aldehyde present in the Kovacs reagent.

It is also noted that the methyl red test is positive by changing the color of the red methyl Indicator from yellow to red due to the ability of bacteria to ferment glucose and produce acid.

The catalase test showed the formation of bubbles resulting from the decomposition of hydrogen peroxide using the catalase enzyme produced from isolation.

The table (3) also shows the inability of bacteria to utilizethe citrate as the sole source of carbon, as the color of the medium remained green and did not change. It is also noticed that it is unable to ferment the cellobiose and its inability to grow in the presence of potassium cyanide, and it is negative for the oxidase test due to its lack of cytochrome c oxidase, and also its inability to produce the β-glocuronidase enzyme, which is one of the most important tests to diagnose *E. coli* O157: H7

The movement test was performed in two methods: the suspended drop method using a combined optical microscope where the movement of the bacteria was clearly observed, and also by the stabbing method of the motility test medium where the growth spread was observed away from the stab line, which indicates the ability of bacteria to move.

The results of the biochemical tests initially showed that the isolate belongs to the E. coli O157: H7 serotype.

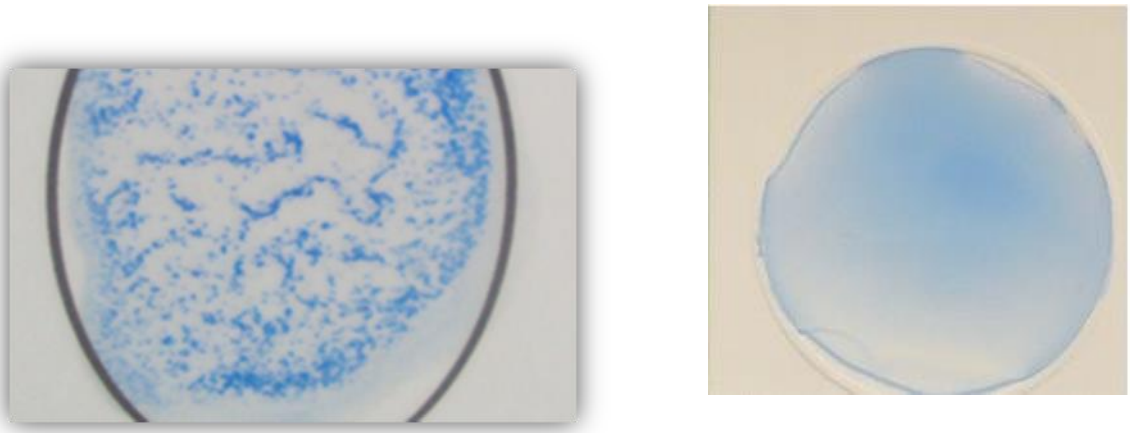
Table (3): Biochemical assays for O157: H7 serotype

testBiochemical	Number of non-fermented isolates of sorbitol that gave a positive result	Number of non-fermented isolates of sorbitol that gave a negative result
Indole test	**1**	**11**
Methyl red test	**1**	**-**
Movement test	**1**	**-**
Oxidase test	**-**	**1**
Catalase test	**1**	**-**
Citrate utilization	**_**	**1**
Potassium cyanide	**_**	**1**
Cellobiose fermentation	**_**	**1**
β-glocuronidase production	**_**	**1**

***Escherichia coli* O157 Latex Test**: the latex test is one of the most important, reliable and easy serological tests for determining the serotype. For this purpose, the control negative, control positive O157 test latex, O157control latex prepared by Oxoid has been used for this purpose.

The results indicate a clear appearance of granularity (agglutination) when using drops of O157 Test Latex reagent (Figure 3A) compared to control treatment (Figure 3B), which indicates the possession of the isolate of the O157 serotype and thus it belongs to the *E. coli* O157: H7

Yousif andHussein(2015) explained that the latex test is one of the most important rapid confirmatory tests for the serotype of *E. coli* O157: H7, which is easy to use because it requires less time and effort. Meiyarasi et al. (2017) indicated that this method is one of the traditional methods of detecting this pattern.



A B

Figure (3): A: The positive result of the latex assay of isolate obtained from the cheese samples, B: the negative result of the control treatment

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الكشف عن بكتريا *E. coli* O157: H7 في الجبن الطري العراقي في بعض مناطق بغداد

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مستخلص البحث:

تم في هذه الدراسة جمع 25 عينة من الجبن الطري العراقي من مناطق مختلفة في بغداد. كان العدد الإجمالي للعزلات 82 عزلة ، وكان عدد العزلات المخمرة للسوربيتول 60 عزلة ، وكان عدد العزلات غير المخمرة للسوربيتول 22 عزلة. شخصت العزلات غير المخمرة للسوربيتول عن طريق التشخيص المزرعي والمورفولوجي والبايوكيميائي ، وأظهرت نتائج الاختبارات البيوكيميائية في البداية أن العزلة تنتمي إلى النمط المصلي *E. coli* O157: H7.

اشارت نتائج اختبار اللاتكس إلى ظهور واضح للحبيبات (التراص) عند استخدام قطرات من كاشف O157 Test Latex مقارنةً بمعاملة السيطرة.

الكلمات المفتاحية: *E. coli* O157: H7 ، الجبن الطري العراقي