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Studying and Determination of Escherichia Coli from for Kinds of Food Samples are Fit for Human Consumption

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Abstract
Twenty plant and animal food samples were collected by from different commercial stores. These samples were placed in a sterilezed plastic bags not to be polluted and were moved to the laboratory in special thermal containers to be tested. The solid samples were processed in the Digester to study each sample separately, while the liquid samples were tested immediately after being diluted. After that they were studied with biochemical tests to identify the type of germ that existed. Results showed that, through these twenty samples the positive samples of the Escherichia coli were (13); (65%) while the negative ones were (7);(35%).
Keywords: Escherichia coli- Food samples-Biochemical tests

1. Introduction

Escherichia coli: is a gram-negative, Anaerobic fermentation, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms) [1], Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts.

The harmless strains are part of the normal flora of the gut [2,3,4], and can benefit their hosts by producing vitamin K [5], and preventing colonization of the intestine with pathogenic bacteria.

In the present [6], If oxygen was absent *E.coli* was switching to fermentation or anaerobic [7]. Cells are typically rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 [8,9]

E. coli stains Gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such as *E. coli* does not damage by penicillin [10].

In 1885, the German-Austrian pediatrician Theodor Escherichia discovered this organism in the feces of healthy individuals. He called it *Bacterium coli commune* because it is found in the colon. Early classifications of prokaryotes were placed these in a handful of genera based on their shape and motility (at that time Ernst Haeckel's classification of bacteria in the kingdom Monera was in place) [11,12,13], A research has been done to study *Escherichia coli* in milk and other dairy products in India, It has shown that out of 135 samples taken 25 were contaminated, with the highest contamination rate being discovered in the samples taken from street vendors (26%) followed by dairy products (20%) and then home-produced milk (6.6%) [14,15], In Malaysia 25 samples of cow meat bought from supermarkets were tested by taking 25 g from each sample and mixing it with 225 ml of Difco for two minutes then incubated at 37 degrees for 4 hours. After that the mixture was diluted with water tryptone (1% tryptone and 0.5% sodium chloride), planted on MacConkey Agar and incubated at 37 degrees for a whole night, From each sample between 20-50 colonies were chosen to be examined, then The results showed that out of 50 samples previously diagnosed with Escherichia coli using Agar EMB; only 40 tested positive for E. coli using differential chemical tests.[16,17], Research has shown that Escherichia coli strain O157: H7causes about 73500 new disease cases and over 60 deaths annually in the united states of America [18], Transmitting this bacterium occurs through drinking contaminated water (waters that have come in contact with animals` fecal matter), swimming in contaminated pools and lakes and eating fruits and vegetables that have been washed in contaminated water, It is important to note that the age and immune system of the person affected by such a disease play a major role in its progress.

Studies have shown that infants aged younger than 5 years are more susceptible to this bacterium, attracting this disease can occur through consuming undercooked meats and burgers, taking immune-suppressing medication and eating on surfaces at service restaurants.[19], It has also been proven by some studies that 4 out of the 8 major causes for attracting Escherichia are undercooked meat and 3 are contaminated drinking water [20]. Until now the biggest cause for Escherichia diseases in Japan has been consuming contaminated radish buds, fruit juices and vegetable salads [21], In 1982 the first case of Escherichia related disease was linked to undercooked cow meat and unpasteurized milk [22], In 1995 the US linked the bacterium *Escherichia coli* to spinach as well as many animals including horses, dogs, mice and cats, it has also been recently shown that 26 chicken samples out of 720 tested positive for *Escherichia coli*, Also the oysters found in contaminated waters off of the coast of France have been tested positive for Escherichia [23-24], In a study done on field mollusks

sampled from a farm in Scotland, the researches were able to find this bacteria in the mucus surrounding the mollusks`bodies [25-26].

This research seeks to isolate the *Escherichia coli* bacteria that are found in the food samples, categorize them and study the bacterial content of the food and how good it is for human consumption with a statistical study to evaluate the results.

2. Research material

Spiral - Petri dishes - pipettes - glass flasks- VRB (Violet Red Bile lactose Agar) medium,The general census Nutrient Agar Pepton test medium, puffer amid extend canthal, Simmons citrate Agar, (MRVP) medium, Indole test - Methyl red test - Voges Proskauer test (VP).

3. Research Methodologies

Different food samples from cheese, dairies, salads, juice, ready food, and meat were collected by from different sources. Such as chicken table (1), After the collecting, the sample has weighted, where 10 g from each sample has taken, the size was completed by Peptone to get 100 g to achieve 1 of 10 dilution, after making the proper dilution for each sample, suitable mediums to discover the *Escherichia coli*, mediums such (EMB), (NA), and MacConkey medium, Then 1 ml of different dilutions were added to the plant on Petri plates, and were incubated For (24) hours on 37C°. degree in reversed condition. next day, the plates were taken out and the transplant results were collected on each medium Eosin Methylene Blue, MacConkey medium, Nutritious Agar, then tests to discover the *Escherichia coli* were done using the methyl red and (MRVP) medium. In addition to, Voges _ Proskauer, Simmons citrate test, and Finally Indole test.

Table 1. the used samples and the time of collecting.

Month	July	August	September	October	November
Number of samples	3	7	4	3	3

4. Result and discussion:

The results of the food samples show:

Table 2. show the dilutions on solid samples:

Sample	Dilution	MacConkey medium	EMB medium	NA medium
Cheese	0.0001	39	Green spangle	80
Halawt AL Jubeen	0.01	0	0	0
Halloumi cheese (1)	0.01	0	0	0
Halloumi cheese (2)	0.01	0	0	0
Chicken sample	0.01	51	Green spangle	100
Salad	0.01	47	Green spangle	50
Meat	0.01	30	Green spangle	50
Shawrma	0.01	9	Green spangle	6
Shanti powder	0.01	0	No spangle	0

Table 3. show the dilutions on liquid samples:

Sample	Dilution	MacConkey medium	EMB medium	NA medium
licorice	0.000001	5	Green spangle	4
Jalab	0.0001	100	Green spangle	45
Tamarind	0.001	5	Green spangle	200
Yoghurt	0.01	5	Green spangle	90
Juice (1)	0.01	0	0	0
Juice (2)	0.01	0	0	0
Juice (3)	0.01	0	0	0
Carbonated water	0.01	26	Green spangle	60
Juice	0.01	80	Green spangle	70
Orange blossom water (1)	0.01	17	Green spangle	100
Orange blossom water (2)	0.01	2	Green spangle	2

Table (2) shows nine solid samples from which five were positive to contain *Escherichia coli* and four negative ones. Haloumi cheese sample was free due to the way of its manufacturing where is put on high temperature kills germs.

Table (3) shows eleven liquid samples from which eight were positive to contain *Escherichia coli* and three negative ones.

The method used to discover *Escherichia coli* is colony form using EMB and MacConkey mediums, Where usually in medical Laboratories the type of colony is diagnosed on the light of shape characteristics without using any other means, The characteristics of the *Escherichia Coli* in this form as shown in figure (1), all Isolates were moved again to EMB medium when the comparison was made, It was clearly that some of the re-subculture purified Isolates after a period of weeks of keeping vin refrigerator, Isolates lost the metallic green spangle which appeared in the initial transplant, the result show the appearance of crimson color on the MacConkey medium because of the *Escherichia coli* fermentation of lactose sugar. Figure (2).

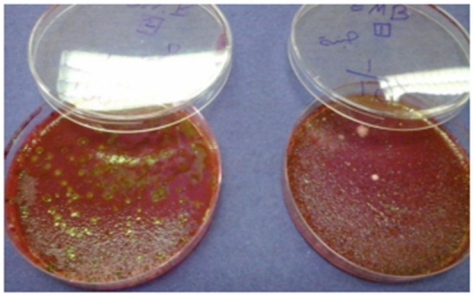

Figure 1. the left and right side of the planted sample of *Escherichia coli* in EMB medium.

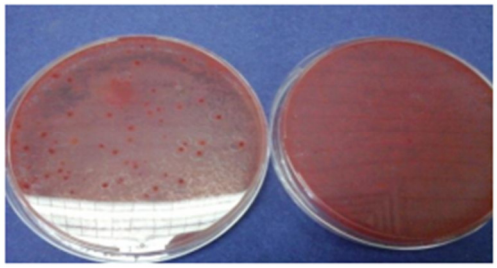

Figure 2. the right planted plate show *a non-Escherichia coli* sample in MacConkey medium while the left side show an *Escherichia coli* sample.

Based on the biochemical differences the results has showed that 13 samples of the 20 isolates were diagnosed to have *Escherichia coli* in the different chemical tests with a percentage of (65%). Table (4). While the remaining seven samples does not has any *Escherichia coli.*

Table (4) the results of tests that show the *Escherichia Coli*.

the Biochemical differentiation test	Indole test	The methyl red test	Voges	Proskauer	Simmons citrate
Escherichia Coli	+	+	-		-

Figure 3. Indol test.

Figure 4. The methyl red test.

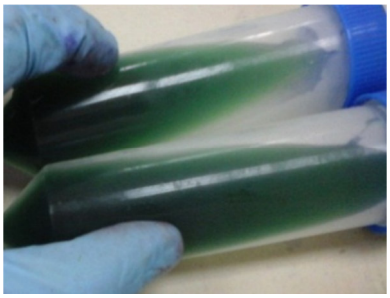

Figure 5. Simmons citrate.

Escherichia coli is characterized as follow: positive rhamnose, saccharose fermentation different from one strain to another, Inositol negativity, Indole production, all strains was negative in (Pro Voges Proskauer duction of the Alosatoian) -

All strains negative in urease - negative in production H_2S negative- Gelatin is not diluted, negative Citrate according to the research of (Barbara et al.2000).

5. Conclusion

The differentiative medium EMB is a good selective differentiative medium and the laboratory workers are keen

in dealing with it, and the *Escherichia Coli* is one of the most obvious colony. Where the common Agar MacConkey has a bigger possibility of error because it depends on dividing the positive Lactose colonies regardless of their size and there is not a *Escherichia Coli's* distinctive spangle.

then the suggested solution which combines between the lower price to the mediums and the high authenticity of PCR is: by using simple differentiative biochemical reactions selected on the light of the resulted error, and which can be prepared easily by using its scientific formula.

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