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RESEARCH ARTICLE

Isolating and diagnosing the *Campylobacter* Bacteria Types from Raw Milk and its Products and its Relation to Children Diarrhea

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Abstract

The study comprised Isolating and diagnosing the *Campylobacter* Bacteria Types from Raw Milk and its Products and its Relation to Children Diarrhea. The study is conducted in the Graduate Studies Lab. In the College of Pure Sciences. The researcher compiled (164) samples from various sources comprising of (40) raw milk samples, (40) local white cheese samples, (40) local cream samples compiled from local markets in Baqubah city during the period of time from (1/11/2017 to 15/9/2018), and (44) samples of feces from children with diarrhea (watery, mucoid, bloody) of ages between (3 months to 5 years) in Al-Batoul Hospital for Maternity and Children. Results of the study exposed that (44) samples, with a ratio of (37.0%), showed negative growth to the bacterial plantation, and (90) samples, with a rate of (62.19%) mirrored positive growth to the bacterial plantation. Moreover, it is founded that (65) samples with a ratio of (51.829%) were *Campylobacter* bacteria as the ratio of isolated bacteria from raw milk reached up to (20) samples with a rate of (50%), (9) samples from local white cheese with a rate of (22.5%) and (8) samples from local cream in a ratio of (20%). It is worth noting that bacteria were diagnosed in terms of ability to live on selective media (Skirrow-Preston) and the second method which is planting on non-selective media (Blood agar, Chocolate agar, Macconkey agar). In addition, the ratios of isolated bacteria from feces samples were, (5) samples of bloody sustenance (11.36%),)19) samples of mucoid sustenance (43.18 %) and (20) watery samples (45.45%). The bacteria were also diagnosed by means of micrography which showed its negativity and positivity towards Gram stein; (92.90%) were negative to Gram stein, while (8.09%) were positive to Gram stain. Furthermore, micrography exposed their negativity to Gram stein and showed its different shapes; Bacillus, Spiral, S-shape and Bird-wing shape. Results showed an increase of bacteria in milk and milk products which included many sicknesses and sitotoxism causes which lead to diarrhea among children. Biochemical and physiological examinations were executed and isolates were oxidase-positive, catalase, urease test, Hippurate hydrolysis test, and growth test within (37^{o} C -42^{o} C) temperatures as well as their tolerance of salinity with concentrations between (1.5 %-3.5%).Isolates were sensitive to Nalidixic acid and resistant to Cephalothin.

Keywords: *Campylobacter (C. jejuni, C. coli, C. hyointestinalis, C. lari), cadF gene, Diarrhea for children.*

Introduction

The raw milk from the food complex composition, containing all he needs the human body from the food Component necessary for a constructive and balanced proportions, Milk contains protein (casein-lactoalbumin-lacto globulin) and milk sugar (lactose) and milk fat as well as vitamins and minerals and rare metals and also balanced quantities in the form liquid easy digestion, acceptable taste and smell, Milk and milk products is appropriate media for the growth and activity of microorganisms, due to the nature of the milk composition to contain a high proportion of water, as well as the presence of milk sugar (lactose) Fermentable and the presence of proteins, salts, vitamins and fats, addition to the pH 6.6 . So all these factors help and make milk appropriate media for the growth and reproduction of microorganisms [1]. Considers the cream cheese of the most food consumed in the cities of Iraq all that the production of local cream and white fresh cheeses are mainly from animal breeders or factories especially

small and directly sold to consumers, so it is expected not to apply the necessary health requirements at the production and exposure to various sources of pollution starts from the marketing and storage and presentation to the consumption, This is because these products are characterized by rapidly it damage is a good media for the growth of bacteria which makes them a means for the transfer of a number of diseases and causing major the number of cases of food poisoning [2]. The cheese industry requires process cleanliness, careful attention by appliances and tools, airspace surrounding with the process industrialization, [3].

While the cream is that part of milk rich in fat and called the local (Qamar Arabs) characterized by its containing to the high thermal energy because of the fat content in addition to other milk components as a protein, sugar, mineral salts, and vitamins. Characterized cream quickly it's damaged and is a good media for the growth of bacteria, which is a source of important food poisoning and disease transmission [4]. Milk is a nutrient medium appropriate for the growth of many microorganisms to fit on many of the carbohydrate compounds and nitrogen.

In addition to the provide a lot of vitamins and various minerals that make it a suitable environment for microscopic organisms that play an important role in Due to vital changes and chemicals that determine the nutritional value and quality cheese and its validity for human consumption and that sometimes lead to many cases of food poisoning to consumers and damage and the corruption of the product.

And Mate [5] in the study conducted by to identify some microbial contaminants local White Cheese fresh and local cream (Qamar Arabs), where results showed a research height of local fresh cheese and cream content of microbiology, which included many of the pathogens of humans and causes food poisoning. *Campylobacter* bacteria belonging to the family of Camplyobactereaceae includes many types of them pathogenic and the other part is nonpathogenic. The name *Campylobacter* is derived from the Greek word "Kampylos," which means curved. *Campylobacter* spp. are non-spore-forming and negative-gram bacteria. They can be spiral, curved or occasionally straight rods, with size ranging from 0.2 to 0.8 μm wide and 0.5 to 5 μm long. Campylobacter may appear as curved rods, "gull wings", S shapes spiral, S-, V-, or comma-shaped forms and may also be found in short or occasionally long chains. As *Campylobacter* cells begin to age, they become coccoid in shape. The cells are highly motile by means of single or occasionally multiple flagella at one or both ends. Extremely rapid, darting motility of comma-shaped cells can be seen with a phase contrast microscope. Ranges along the flagella (3-2) times as much as the length of the bacterial cell.

These microaerophilic organisms grow best in an atmosphere containing 5 to 10% oxygen and optimum temperature for their growth ranges from 30 to 42°C. Colony morphology is quite variable, with a thick translucent white growth to spreading film-like transparent growth which can be visible on the plating media within 24 to 48 hours of incubation. *Campylobacter* does not ferment carbohydrates and usually, obtain energy from amino acids or tricarboxylic acid cycle intermediates. Typical biochemical reactions include negative methyl red and indole production. Most species reduce nitrate and are oxidase-positive but only C. jejuni is hippurate positive, Catalase test some of them will be positive for this test and others will be negative [6]. It also is sensitive to antibiotic nalidixic acid and resistance to antibiotic Cephalothin. Are some species belonging to the genus *Campylobacter* is one of the most frequently reported causes of acute bacterial gastroenteritis.

Significant variations in incidence rates have been observed between different countries, *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as one of the most frequent causes of acute diarrheal disease in humans throughout in the developing and developed Countries [7]. Diarrhea disease and its complexities is a major cause of death in children, especially in the developing countries, as it is secondary and the most common reason of death for children under five years of age in the world [8]. The causes of diarrhea are due to the presence of different types of pathogens differ in developed and developing countries that may

be causes of a virus or bacterial or parasitic infections or other a mixed between these species [**9**]. The genus *Campylobacter* includes many species namely (*C. jejuni and C.coli and C. lari and C. fetus and C. hyointestinalis*). Species of pathogens that have come from contaminated food, causing diarrhea, the also caused other injuries outside of intestinal diseases of secondary [10].

The most important symptoms of this bacteria are Watery diarrhea, Bloody diarrhea, and stomach cramps cause gastrointestinal infections as well as post-infection manifestations, e. g Guillain-Barre syndrome or reactive arthritis [11]. Future studies trending toward comparing the quality of the gene by using the method (PCR- RFLP) and (ALFP) standard serological methods electrophoresis is the usual way in the laboratories of Public Health [12].

Several the previous studies have references to the use of amplification for the detection of these bacteria in faecal samples through the use of target genes (*ceuE* gene - *16SrRNA* gene - *glyA* gene - *23SrRNA* gene and *cadFgene*) where the bacteria produce a lot of virulence factors such as proteins the outer membrane and endotoxins and other factors that increase the pathogenesis of bacteria [13]. Due to the importance of bacteria Campylobacter bacteria, therefore, this study aims to:

- Isolate and identification species of *Campylobacter* bacteria causing diarrhea (*C. jejuni - C. coli - C. hyointestinalis. - C. Lari*) of raw milk and its products.
- Knowledge of the relationship of these bacteria cases of diarrhea in children.
- The use of PCR technology for the detection of virulence gene that causes diarrhea *cadF* of bacteria *Campylobacter jejuni.*

Materials and Methods

Isolation and Diagnosis

Sample Collection

164 samples were collected from various sources including 40 samples of raw milk, 40 a sample of white cheese, local bread and 40 a sample of the local cream "Qamar Arabs" and placed in sterile tubes from the local markets in the district of Baquba during the period from (1/4/2018) to (15 /9/2018), With the carefully that the samples are not to pasteurization, sterilization, and boiling. And collected from (44) a sample of the faeces children with diarrhea (watery, mucoid, bloody) of ages between (3 months to 5 years) in Al-Batoul Hospital for Maternity and Children then the samples directly sent to a laboratory to the College of Graduate Studies using a box of cork containing pieces of ice to maintain the temperature grade samples**.**

Campylobacter ssp. Isolation

Isolation Raw Milk and its Products

Taking the (1 ml) of raw milk and put the sample in a tube sterile containing (9 ml) of a sterile saline solution (dilution 1/10 [wt/vol]), For homogeneous contents and gets a homogeneous distribution of bacterial cells by using Vortex Mixer and, then transferred from the tube (1 ml) by pipette into a new sterile tube containing (9 ml) of a sterile saline solution and continue working in dilutions, Then take all the dilution (0.5 ml).

Are grown in dishes containing the media (Preston Campylobacter Blood- Free Selective) and (Skirrow Selective Supplement III).Then leave the dishes at room temperature for a period of (5 minutes) and the dishes were incubated in the fewer oxygen of conditions Microaerophilic in an anaerobic incubator at a temperature (37^{o} C) for a period of (24-48 h). It is then examined the dishes after a period of incubation in order to be described colonies, Subculturing from one individual colony was repeated until pure culture obtained. In the next step, a white single colony was selected and their morphological characterizations were studied after gram staining under the microscope.

Were prepared cheese emulsifier and local cream where weight (1g) of the sample transported after cutting by using sterile vine tool (in the oven for 2h) to the tube sterile containing, (10 ml) of the dilution solution (sodium citrate 1 -2% g of peptone). And then mixing the solution well by using Vortex Mixer machine for two (5 min) and at high speed and then leave the solution for a period of (15 min) in order to regain its vitality microbiologist and stirring is continued slowly to ensure the homogeneity of the solution process. And it took a

quantity of emulsifier solution by cotton swab carrier or Loop then inoculation cultures media are used is (Preston Campylobacter Blood- Free Selective),(Skirrow Selective Supplement III) ,The dishes were incubated in conditions of low oxygen conditions Microaerophilic in anaerobic incubator at a temperature (37 C) for a period of (24-48 h). It is then examined the dishes after a period of incubation in order to be described colonies. Sub culturing from one individual colony was repeated until pure culture obtained. In the next step, a white single colony was selected and their morphological characterizations were studied after gram staining under the microscope.

Isolation from Fecal Samples of Children

It was collected 33 fecal samples of children with watery or bloody diarrhea by using cotton swabs and placed in a sterile containing tubes normal saline solution and ages between (3 months -5 years) of Batool maternity and children hospital in Diyala province during the period (1 /11/2017) to (28/12 /2017) and recorded the information on the patient from (Age - Gender - date of taking the sample) and then transferred the samples directly to the lab at the College of Graduate Studies by using the box cork containing pieces of ice to keep the degree of heat samples for the purpose of conducting examinations and transplantation and diagnosis of the study samples.

Take swabs from a fecal sample and placed in normal saline solution then placed tubes in the centrifuge by speeds (3000 rpm) for a period of (30 min) and then taken from the filtrate by cotton swabs and inoculated with the media of Agar Columbia containing antibiotics and the blood of humans and growth promoters. (Skirrow Selective Supplement III) The dishes were incubated in conditions of low oxygen conditions Microaerophilic in an anaerobic incubator at a temperature (37 C) for a period of (24-48 h). It is then examined the dishes after a period of incubation in order to be described colonies. Sub culturing from one individual colony was repeated until pure culture obtained. In the next step, the white single colony was selected and their morphological characterizations were studied after gram staining under the microscope. Isolates were diagnosed according to the Phenotypic and microscopic by characteristics by using biochemical tests according to a [14].

Molecular Identification

After of the total DNA extraction of the bacterial colony by Mini DNA Bacteria kit Bioneer (Korea) PCR was performed to amplify a 400 bp region of *cadF*, a genus-specific virulence gene of *Campylobacter*, using the following DNA primers (Table 1). This segment of *Campylobacter* DNA corresponds to sequences described by [12]. The PCR reaction mixture (25µl) consisted of 6µ extracted DNA, 3µ PCR reaction buffer, 50 pM of *cadF* forward and reverse primer, 0.25mM concentration of each dNTP, and 1U of Taq DNA polymerase. PCR run. Cycling conditions used were: pre-heating at 94°C for 4 minutes, denaturation at 94°C for 1 minute, annealing at 47°C for 1 minute, extension at 72°C for 7 minutes, and a final extension at 72°C for 5 minutes.

Thirty-six cycles were performed using the thermocycler (Eppendorf, Germany). Amplified PCR products were electrophoresed on 1% ethidium bromide-stained agarose gel, along with a molecular weight marker (2000-bp DNA ladder, Bio-basic Inc.). The electrophoresis was carried out at a constant voltage of 100V for 1h, and a band of 400-bp was taken to be positive. The bands in the gel were photographed under UV transillumination (Figure).

Table 1: Sequences of the *cadF* genes Primers used for PCR Amplification

Primers	sequence (5´ to 3´)	Size (bp)	Reference
cadF **–F**	**-"5-TTG AAG GTA ATT TAG ATA TG-3"**	**400**	**(13)**
cadF **-R**	**"5-CTA ATA CCT AAA GTT GAA AC-3"**		

Results Discussion

Morphological and Biochemical Characterization of Isolates

In the present study, 120 *Campylobacter* isolates and by (73.17%), were isolated from Raw Milk and its Products and fecal children, While (44) samples were negative bacterial growth for transplantation, study included (40) samples of raw milk and (40) a sample of White Cheese local and (40) a sample of the local cream "Qamar Arabs

were grown on media of selective is (Preston campylobacter blood-free) and Columbia blood base agar. The percentage of raw milk contaminated with bacteria *Campylobacter* (50%) either contamination of the local cream proportion (22.5%)while, the local white cheese contamination percentage (20 %).90 sample (73.17%) positive growth for transplant bacterial isolate which 65 isolation (52.84%).

Table 2: Percentage of isolates positive to culture media isolated from Raw Milk and its Products

The type of sample	The number of samples tested	positive examination
Raw milk	40	%50
Local freesh cheese	40	%22.5
Local cream	40	%20
Total	120	%33.3

The study also included 44 faecal samples of children with watery or bloody diarrhea (20 watery, 19 mucoid, 5 bloody) and ages (3 months - 5 years), which grew on of selective media (Skirrow and Preston), Where the results of the current study showed that (11.36%) of bloody diarrhea (43.18%) of watery diarrhea and (45.45%) of mucoid diarrhea.

Table 3: Percentage of isolates positive to culture media isolated from fecal samples of children

The type of fecal	The number of samples tested	positive examination
Bloody	5	%11.36
Mucoid	19	%43.18
Watery	20	%45.45
Total	44	%100

The study showed by using gram stain, negative gram bacteria (92.90 %) while the positive gram bacteria stain (8.09%). The different *Campylobacter isolates* were confirmed on the basis of the method described [14].Shape, gram staining and the presence of spores. *Campylobacter* may appear as curved rods, "gull wings", S shapes spiral, S-, V-, or comma-shaped forms, non-spore–forming. These cells appear in the form of symbols (S) and that's when two of the cell associated with each composed a short series of these cells appear in the old colonies spherical or circular shape (Gull _ Winged). These bacilli spherical Coccoid in the old colonies, this change occurs as a result of the permeability of nutrients or because of a high percentage of oxygen, which leads to a change in cell envelope is the spiral shape of the genus *Campylobacter*, the most distinctive characteristics distinguish them from the rest of enteric microbes [15].

Table 4: Biochemical tests used to identify *campylobacters.*

Characteristic	*C. hyointestinalis*	*C.lari*	*C. coli*	*C. Sub sp doylie*	*C. jejuni*
. Observation					
Growth at (37C)	+	+	+	+	+
Growth at (42C)	+	+	+	+	+
Nacl at (1.5% - 3.5%)	-	+	+	-	-
H2S- TSI	+	-	-	-	-
Catalase	+	+	+	-	+
Oxidase	+	+	+	+	+
Macconkey agar	-	+	+	-	-
Hippurate hydrolysis	-	-	-	+	+
Ureas agar	+	+	+	+	+
Resistance to Nalidixic Acid	R	R	S	S	S
Resistance to Cephalothin	R	R	R	S	R

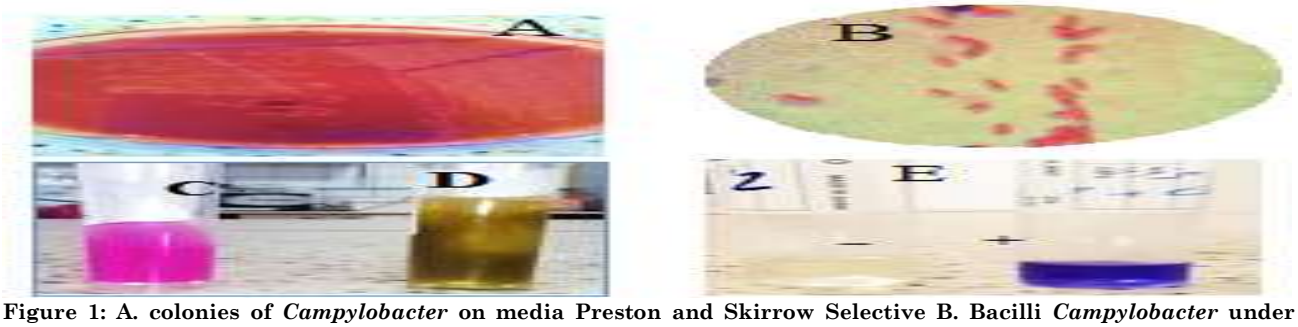

Figure 1: A. colonies of *Campylobacter* on media Preston and Skirrow Selective B. Bacilli *Campylobacter* under microscope, C. Urease test, D. product of H2S test, E. Hippurate hydrolysis test

Tested 9 samples of the bacteria Campylobacter bearing sequences (5-10--12-17-20- 24- 26 -27-29), as selected samples grown on Preston and Skirrow Selective, where they have selected these samples to detect for susceptibility of bacteria to produce genes responsible for virulence factors, including the virulence gene *cadf*. Using a technique (PCR) and depending on the Primers specialized gene *cadF* and equipped with by a company (Bioneer \ Korea) according designer sequence by [13], was detected products extracted DNA bacteria by using electrophoresis on agarose gel technology and use Spectrophotometer a device for measuring the purity where the purity of the samples ranging from (2-1.8).

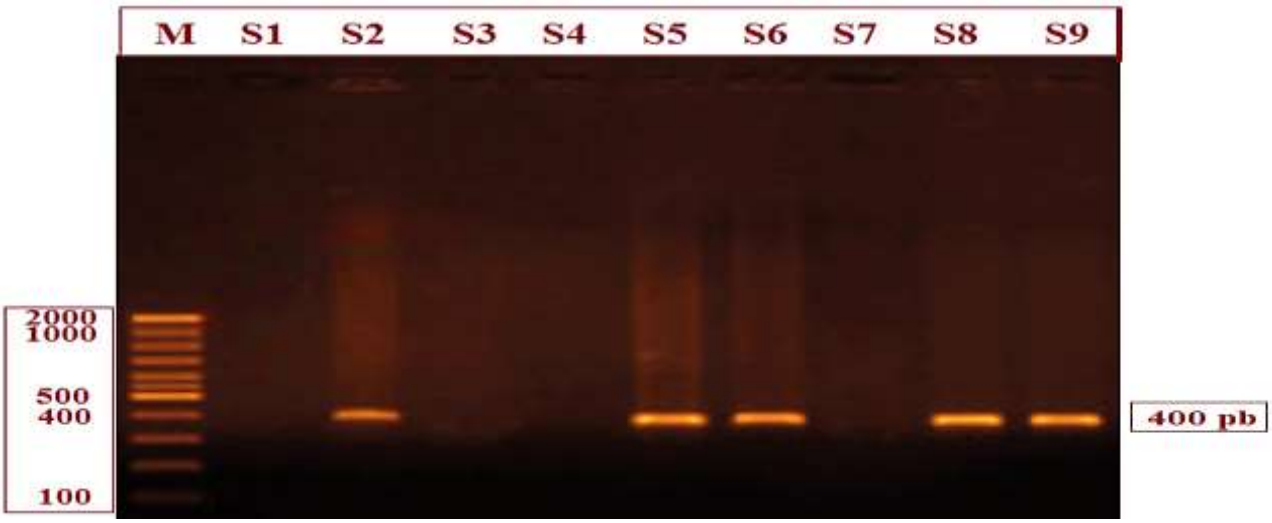

Figure 2: The result of the amplification of *cadf* gene on the *Campylobacter* sample isolated from the Raw Milk and its Products and fecal children. Line 1, size Marker 100 to 2000 bp, Line S2, ,, , 5,6, 8,9,positive isolates, Line S1,3,4,7 negative isolates amplification by PCR

Discussion

The current study included isolate and identification species of bacteria *Campylobacter* from raw milk and its products and its relationship to cases of diarrhea in children, has been observed contamination of raw milk samples and products for these bacteria where the raw milk contamination percentage (50 %) and the percentage of the white local cheese contamination of (22.33 %) while, the local cream "Arab Qamar" contamination percentage (20 %). This explains the presence of bacteria on the basis of the use of raw milk contaminated with large numbers of bacteria, which moved him from a cow infected or carriers of the bacteria or from working in milk production, as well as the lack of thermal treatment to ensure the arrival of this bacteria to raw milk and This agrees with [4].

Also, dairy products including local cream and local cheese that may occur contamination after the pasteurization process of the reason is due to the poor quality of raw milk user, may resort some of the countries to the manufacture certain types of cheese from raw milk or milk treated thermally at low temperatures that the use of degrees low temperature leads to increase the percentage of microorganisms in milk intended for the manufacture, because low temperature will not eliminate microorganisms pathogenesis found in milk, which needs to temperatures (72 ° C) for a period of 15 seconds at least, and This agrees with [16]. Explained the two researchers [17] food and animal role in the infection of these bacteria to humans as eating contaminated food especially unpasteurized milk and drinking water is not sterile and poultry as well as to touch the with domestic animals, particularly chickens, cats, and dogs can lead to the infection of these bacteria.

The present study included isolate and identification species of bacteria *Campylobacter* from feces of children with diarrhea aged between (3 months -5 years) this corresponds with the data in the Arab Gulf states such as Saudi Arabia, Kuwait and Bahrain for the age of children whose have isolated them bacteria were more cases for ages under 4 years [15,18].

The Note feces kind into whether mucous or bloody or watery, where the bloody feces ratio (11.36%) is caused is due to the multiplication of bacteria inside the inner

layer of the intestine and secreted toxins cellular which lead to bloody inflammation, while the percentage of feces mucosa (43.18 %) is due caused to the presence of mucus or pus, while the watery feces ratio (45.45%) is due caused by a viral infection or a bacterial or allergies or intolerances kind of foods such as dairy products, and this is due to apparent presence of other species bacteria or parasites such as *Entamoeba histolytic* this agrees with [15,19].

In our study sample was limited to children ages (3 months - 5 years) through the collected information shows that dependence on mixed feeding consti Without a high nutrition type ratio and this explains the occurrence of diarrhea because of the possibility of contamination when preparing artificial feeding, and the level of awareness and cultural for the mother and the environment It is based on an important role in increasing the percentage of infection and This agrees with [20].

Diarrhea is caused by the bacteria *Campylobacter* is concentrated among children and a few cases of an adult. with depending on the process staining gram it was found that the percentage of positive gram bacteria was (8.09%) is caused is due to the presence of bacterial pathogens Other positive gram bacteria such as *Streptococcus* and *Staphylococcus*.

The percentage of negative gram bacteria was (92.90%) and this is due to the presence of bacteria that may be contaminated with the feces or Flora included these types *Enterobacter- Pseudomonas* that this pollution is caused by blood use in a dish transplant as it provides nutrients and allows for the growth of other bacteria species, and therefore it is difficult to get pure colonies of bacteria this is agree with [15, 19, 21].

And that the use of blood volume (5.7%) (V / v) in order to extinguish the toxicity of oxygen compounds such as hydrogen peroxide when exposed to light, while the ability of bacteria to the haemolysis, it is not haemolysis. The modern research refers to that it caused two types of haemolysis, alpha, and beta, especially species *C. jejuni* this agrees with [15, 22]. Results showed cultures media positive and negative, after bringing a fecal sample by Normal Saline can be described colonies which was shown after the transplant on selective medium (Columbia blood base agar) and (Preston Campylobacter blood - free medium) containing a human blood and antibiotics and growth stimulants the previously mentioned, after incubation temperature of (42 ℃) for a period (24-48 h) in a few oxygen conditions where the colonies mucosa and gray color and similar to small water droplets and sticky this is consistent with [23].

Appeared during microscopic examination of different shapes differ according to the causes of participation of diarrhea, the shapes that appeared are the bacilli-shaped (S) when it is associated bi-cell with each other or by shape the wings of a bird, whereas the colonies in the old plantations after 48 hours spherical shape due to permeability food or cells exposed to a high concentration of oxygen, which leads to a change in the growth of bacteria, this is the change in the shapes cells to bacteria *Campylobacter* of phenotypic characteristics and distinctive this agrees with [20].

It is well known that the bacteria *Campylobacter* distinguished by a lack of effectiveness and biochemical though tests were used to confirm, Though tests were used to confirm (Wihtout, 2010; Al- Sibahee, 2010). The test results showed that all isolates are positive test oxidase, either Catalase the test of all types bacteria *Campylobacter* positive for this test except *C.jejuni Sub - dolyei* are negative for this test can also distinguish them by their sensitivity to Cephalothine who uses the media selective isolated.

This agrees with [24]. And she gave the isolates positive test result Hippurate hydrolysis through a be violet color, This is a test of the important diagnostic characteristics of the bacteria *C.jejuni* is its ability to Hippurate hydrolysis and this distinguishes it from the species *C.coli* inability to Hippurate hydrolysis This is agreed with [25].

The urease test has given positive bacteria result of this test through the media color change from yellow to pink This demonstrates the ability of bacteria to produce a urease enzyme This disagreed with [15, 20]. In testing the product of H_2S in TSI media it is negative for this test does not produce H_2S except species *C. hyointestinalis*, where studies indicate that

this species is the only positive for this test [24, 26]. The growth test temperature (37 C° - 42 C°) this is one of the important diagnostic characteristics between the types of bacteria *Campylobacter* [27]. The growth test on the MacConkey agar, bacteria will not grow it except species (*C. coli - C. lari*) both of which are growing on the MacConkey agar this is agree with [24]. In testing motility Bacteria animated polar possession flagella from one side or on both sides of the cell, and movement fast Cork Screw -like motility is one of the diagnostic characteristics seconds after the spiral shape of the bacteria *Campylobacter*, which distinguishes it from other Enterobacteriales [28].

The sensitivity test have emerged resistance to Cephalothine and sensitivity of isolates to Nalidixic Acid, This is agree with [15, 19, 28]. The sensitivity to Nalidixic Acid is one of the diagnostic characteristics of the bacteria *Campylobacter*, but at the present time some complications appeared due to the interference that occurs in increasing resistance to this antibiotic for strains of bacteria showed isolates the current study, all of which are sensitive to Nalidixic Acid This is agree with [29].

The growth at Nacl test (1.5% - 3.5%) This isolates appeared their ability to grow in the sodium chloride concentration (1.5% - 3.5%) so these isolates were positive for this test, which date back to the species (*C.coli - C. lari*), has references sources to the inability of the species *C.jejuni* of growth by concentration (3.5%), where they are sensitive to salinity, [30]. They are negative in the Methyl red tests, vox Proskauer tests, Citrate utilization tests and indole forming (Table 4), because they do not possess the enzyme Altrepettovanez and their inability to metabolize sugar and use Citrate utilization as Carbonia source for the production of energy This is agree with [31].Then, Agarose gel electrophoresis of PCR products using *cad F* primers, along with a molecular weight marker (2000-bp DNA ladder). The electrophoresis was carried out at a constant voltage of 100V for 1 h, and a band of 400-bp was taken to be positive. The bands in the gel were photographed under UV transillumination, Where it was noted the emergence one band from all the wells and with the same size is expected to gene *cadf* is (400bp), after exposing the gel to Ultra Violet light figure(1).

And when compared with with the results of the [13].The current study, showed that (4) samples of the bacteria *campylobacter* was negative for examination (44.4%), while (5) positive samples for testing, which contains a gene virulence *cadf*, cadf gene is virulent that Present on the outer membrane of bacteria that help this gene on the adhesion and invasion of bacteria with host cells process and thereby increase the pathogenesis of the bacteria which causes the occurrence of diarrhea in children, and this is agree with [13].

The Gene *cadf* Present in bacteria *C. jejuni* be similar to gene *ompA* the Present in bacteria *E.coli*, which operates through the formation of channels in the outer membrane of the bacterial protein, are these channels with diameters wide, which allow the passage of large molecules such as peptides and the rules of the small nucleotides into lumen plasma which is characterized by the outer membrane of bacteria *C. jeuni* as a molecular weight of between (41. 000-45.000 KDS)., which consists of a large band single protein, this protein extends through a membrane of on the bacterial cell surface, Addition to its association with Peptidoglycan present the inner surface of the outer membrane this is agree with[32,33].

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