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Isolation And Identification of Campylobacter Coli _Campylobacter Lari from Humans, Local Milk, and Milk Products Using Classical And Molecular Techniques In Karbala Province.

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Abstract

This study, which ran from October 2022 to the end of March 2023, examined the frequency of Campylobacter species in samples of human, milk, and milk products in the province of Karbala. as well as the detection of the antibiotic sensitivity of these isolates. A total of one hundred (100) samples were collected systematically, including 100 samples from children at the General Children's Hospital in Kerbala (Iraq) who were experiencing diarrhoea, fever, and abdominal pain, as well as 50 samples of raw milk and 50 samples of milk products from various local shops and farms distributed throughout the province of Karbala. In order to identify the pathogen, samples were promptly inoculated into enriched and subcultured on selective media. On Campylobacter agar base selective medium, Campylobacter spp. Colonies showed up as tiny; mucoid colonies frequently generate separate colonies that are flat with uneven borders and non-hemolytic at 24 to 48 hours. These colonies are typically greyish in appearance, while some must be creamy grey. The polymerase chain reaction method identified ten isolates out of 150 samples (6.6%) as Campylobacter species.

The ten isolates were subjected to sequencing to detect Campylobacter species, which found two species (*C. coli _ C. lari).* Results showed that Campylobacter spp. Out of eight positive isolates, *campylobacter coli* recorded 8 (80%), while c*ampylobacter lari* recorded (20%) positive results. Campylobacter ssp displayed 100% sensitivity to azithromycin, tetracycline, ampicillin, gentamycin, and imipenem in an antibiotic susceptibility test using the disk diffusion assay. Due to Campylobacter's vulnerability to the aforementioned antibiotics, frequently employed as the preferred treatment for campylobacteriosis, the resistance pattern of this bacteria shown in this study is highly concerning. These levels of incidence and resistance call for more investigation and effective defences because they could pose health dangers to the public.

Key words**:** Campylobacter coli & campylobacter lari, infection rate, Milk& milk product, Antimicrobial susceptibility, Kerbala, retail points

Introduction

Campylobacter is a zoonotic tiny curved or S-shaped gram-negative bacterial disease. It is widely acknowledged as the most common source of bacterial milk-borne illnesses worldwide. Since its first taxonomic validation, the genus Campylobacter has grown to include a number of important human and animal diseases. Throughout the previous decades, it has been the most often isolated pathogen in outbreaks in both developed and developing countries. [1]

Campylobacter spp. can infect humans when consumed or handled in foods, including raw or undercooked meat or poultry, raw milk, and dairy products. [2]. Dairy products are thought to be the primary source of Campylobacter infection in people as they are the top item connected to outbreaks of the disease.[3]

The epidemiology of Campylobacter infection varies greatly across industrialized and underdeveloped nations. In undeveloped countries, campylobacter enteritis has no preference for seasonality, but campylobacteriosis epidemics occur in industrialized countries in the summer and fall [1]**,** [4]**.**

The primary outcome of exposure to Campylobacter species through food intake, which is typically diagnosed as gastroenteritis, is campylobacteriosis, a zoonotic infection. [5] According to reports, certain Campylobacter species have the ability to attach to and attack human intestinal epithelial cells using their flagellum, which reduces the capacity of the intestinal barrier, produces toxins, and purposefully inhibits immunological responses. [6] Abdominal pains, diarrhea (usually bloody), vomiting, headache, nausea, dizziness, lethargy, and fever are only a few of the many symptoms. [7] Along with the burden of illnesses brought on by these bacterial pathogens, the emergence of antibiotic-resistant Campylobacter strains is another cost to public health, which may be worse in developing nations where the use of antimicrobials is generally unregulated.[8-9].

This study aimed to determine the prevalence of infection and the molecular identification of Campylobacter coli and Campylobacter lari in human, milk, and milk products in Kerbala retail locations.

Material and method

1. Study design and Sample collection

In order to gather a total of 150 samples from human, milk and milk products, a cross-sectional study was conducted. These samples were collected from various locations throughout the province of Karbala, and they were then grown in appropriate conditions in accordance with industry-accepted guidelines for the growth and identification of bacteria [10]. The initial bacterial isolation procedure was then carried out using campylobacter agar, one of this organism's unique and particular culture media, and propagation was then carried out.

2.Campylobacter Isolation and identification

Collected samples were promptly inoculated onto C&S modified Carry Blair transfer media, then cultivated on selective agar (campylobacter agar enhanced with 5-10% blood), supplements added, and incubated in an anaerobic jar for 48 hours at 42°C under microaerophilic conditions. Gram staining was used to confirm the suspected colony, which was then studied under a microscope. Biochemical testing (oxidase, catalase, and indoxyl hydrolysis) was also performed [11].

Finally, the positive results were confirmed using molecular methods (PCR), and 1 ml of new 24-hour culture broth (Genaid/Korea) was made and utilized directly for DNA extraction in accordance with the manufacturer's instructions. Infection with Campylobacter jejuni and Campylobacter upselienes was verified using the polymerase chain reaction test. Campylobacter ssp sodA gene primer was detected using oligonucleotide primers, forward primer GGATGACACTTTTCGGAGC and reverse primer CATTGTAGCACGTGTGTC, which resulted in amplified 812 bp PCR products. Which were employed to determine whether these bacteria had infected the samples, and the steps with these species-specific primers were carried out as stated by [12] Initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension 72°C for 1 minute, and final extension 72°C for 5 minutes [11]. All positive isolates were transferred to Macrogen for Sanger sequencing. SnapGene version 5.2.5 (www.ncbi pgene.com) was used to manually examine and trim the invA gene's nucleotide sequences for quality.

3.Antibiotics susceptibility testing

In accordance with CLSI recommendations, Campylobacter isolates' susceptibility to twelve antibiotics was evaluated using the modified Kirby-Bauer disk diffusion method. [13]. The widths of the inhibition zones surrounding antibiotic disks were interpreted using the CLSI-published Enterobacteriaceae breakpoints. The 12 antibiotics that were tested and applied to the agar surface were azithromycin (15 mg), ciprofloxacin (5 mg), ceftriaxone (30 mg), nalidixic acid (30 mg), gentamicin (10 mg), penicillin (10 mg), erythromycin (10 mg), tetracyclin (30 mg), imipenem (10 mg), ampiciline (10 mg), amoxicillin (25 mg), and streptomycin (10 mg). A zone of clearing following the incubation period indicated the presence of resistance to ciprofloxacin, gentamycin, and impipenem[1].

4.Statistical Analysis

SPSS (version 21) was employed as the statistical program for this study. Chi-square scores were analyzed to identify disparities within the same group. Five percentage points were deemed to be a significant change (P 0.05) [14].

Results:

1.Culture and biochemical test

A Campylobacter spp. Colony that has been It appears as a tiny, mucoid colony that is usually greyish in appearance, yet some colonies must be creamy grey when successfully isolated on Campylobacter agar base selective medium. Additionally, moist and slightly elevated, these colonies usually develop distinct, flat, non-hemolytic colonies after 24 to 48 hours and have uneven borders.

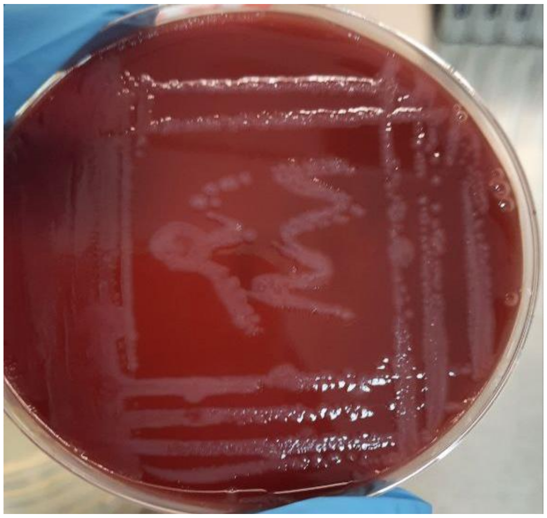

Figure (1): campylobacter colony morphology on campylobacter base agar with blood.

2. Campylobacter spp. prevalence based on sample sources

2.1 Campylobacter species prevalence in milk and milk products

Campylobacter spp. was found in 12% of the 50 milk and milk product test samples. (Table 1); using statistical reasoning, it is predicted that 2% of raw milk and 5% of milk products in the holy city of Karbala are contaminated with Campylobacter species. Regarding the prevalence of Campylobacter, there is a significant difference between milk and milk products (2=o.971, p=0.808).

Table (1): prevalence of *campylobacter spp.* of milk and milk product

TYPE	NO .sample	Positive	percentage
Raw milk	50	4	8%
Cheese	20	1	5%
Cream	10	0	0%
Yogurt	20	1	5%
Total	100	6	6%
Statistical analysis	Chi square= 1.046 *P=0.003*		

The current results show a relationship between the presence of bacteria and the kind of sample, where the findings indicated that milk did significantly differ from milk-derived products. (p-value ≤0.05).

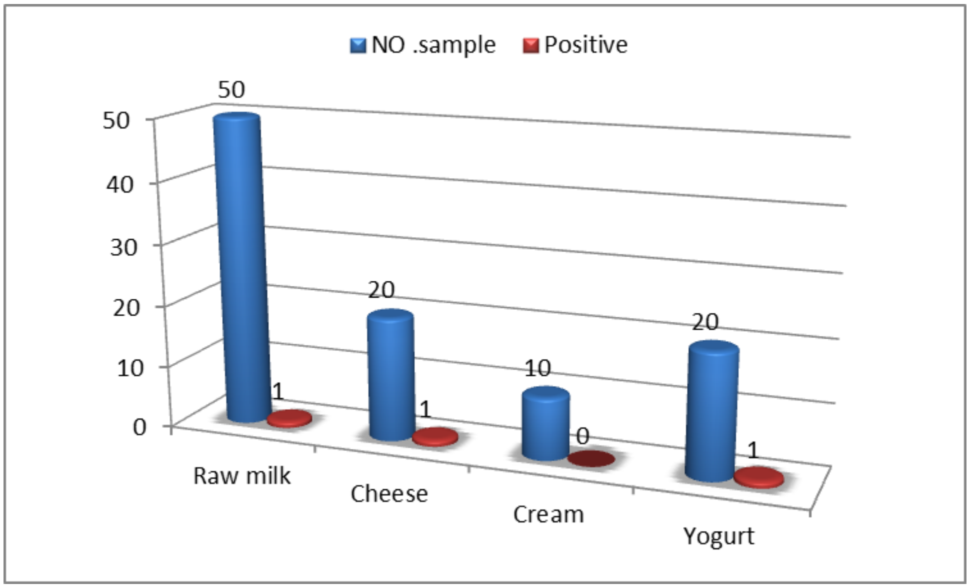

Figure (2) infection rates of *Campylobacter spp.* In milk and milk product.

2.1.1 Prevalence of Campylobacter spp. According to the Months:

The months from which Campylobacter spp. illnesses were more common in our analysis were those from October 2022 to March 2023, as indicated in figure (2).

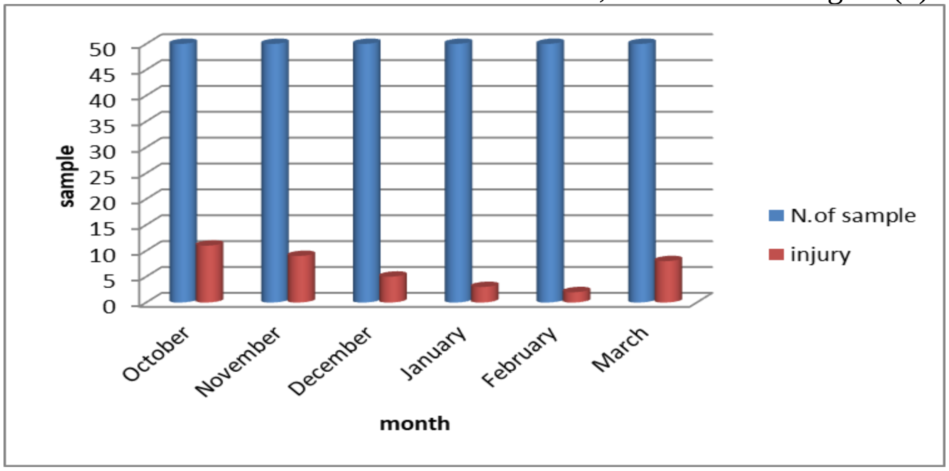

Figure (2): distribution of campylobacter spp. according to month

The distribution of campylobacter spp. by study month revealed significant differences (p0.05), with the lowest incidence of isolation occurring on February 2 (4%) and the maximum percentage of positive results occurring on October 6 (12%).

2.2 prevalence of Campylobacter spp.in Human Stool Samples.

The prevalence of Campylobacter spp. Infection in humans was (4%), based on a total of (50) stool samples collected from infected children who had diarrhea. Some also had other symptoms like fever, colic, and vomiting at educational children's hospitals in Karbala City, was (4%).

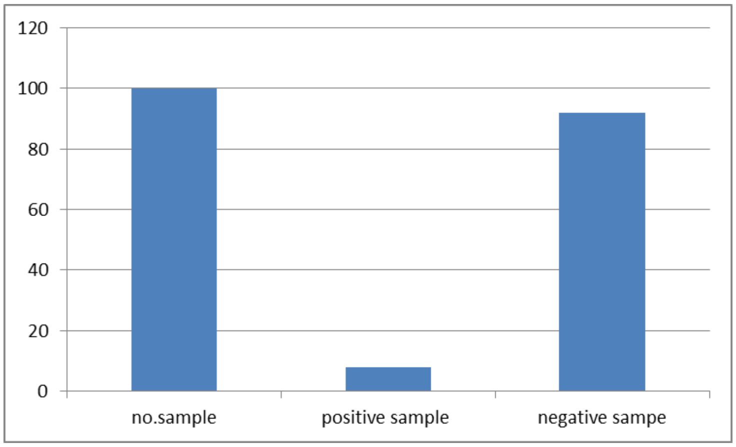

Figure (3): prevalence of Campylobacter spp. in Human Stool Samples

2.2.1: Distribution of Campylobacter isolates according to age:

In the current study, out of 2 (4%) positive results, given the prevalence rate of Campylobacter spp. Infections are highest in children under the age of five and decline with increasing age; there is a significant ($p \leq 0.05$) association between age and infection. There was a significant difference between the percentage of age groups.

Table (2): prevalence of Campylobacter spp. in stool samples from patients, based on age.

Age rang (years)	Number of samples	Number of positive culture	Percentage%
2 month-1year	23	3	13%
1-5 year	19	1	5.2%
5-10 year	8	0	0%
Total	50	4	8%
Statistical analysis	**Chi square =1.765** ***p≤0.05***		

3. Molecular identification

3.1. 16S rRNA gene PCR-based molecular identification

The molecular test used for identifying and confirming *Campylobacter* as a genus by PCR technique, the culture appeared in 15 suspected samples. However, the PCR gave 10(6.6%) positive isolates showed to be *Campylobacter.*

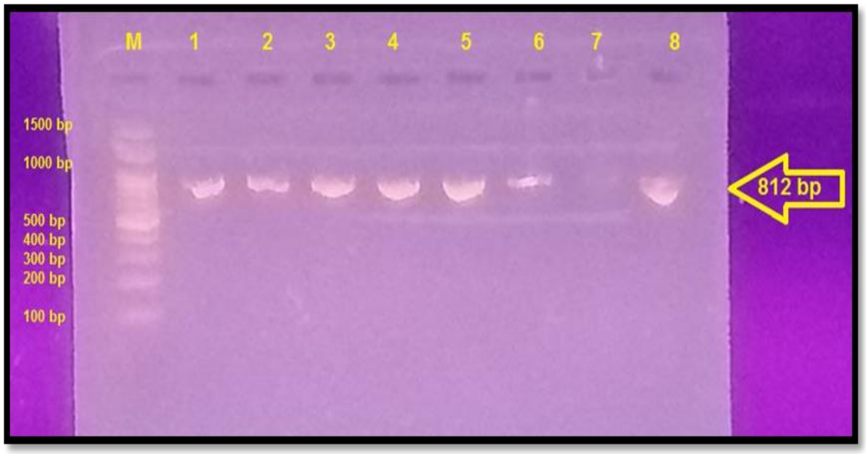

Figure (4): PCR findings for the detection of the 16S rRNA gene unique to the Campylobacter genus on an agarose gel stained with Red-Safe (90 volts/42 minutes). Ladder in Lane M.1500 bp of safe-green Selective DNA Marker; Lanes are labeled with strain identifier codes. Lanes 1, 2, 3, 4, 5, 6, and 8 demonstrate successful results using the 16S rRNA gene (812 bp).

3.2 Typing by Sequence Method

3.2.1 Sequence Typing of Partial 16S rRNA Gene from Campylobacter spp

The molecular characterization of Campylobacter involved the 16S rRNA gene. For Sanger sequencer sequencing, the amplicons from the separated samples were sent to MACROGEN®. When compared to the National Center for Biotechnology Information (NCBI) archives, high-quality nucleotide sequences (forward or reverse) were discovered, and they were categorized using bioinformatics tools and algorithms created specifically for this kind of research.

The isolates were considered the first to be recorded in the Genome Bank and were given special numbers, as indicated in Table, as a result of the results, which showed a 100% match rate with the world's isolates [3].

Table (2): The recorded isolates were considered in the Genome Bank

No.	Analysis	Accession number	Code	Source
1	C. Lari	OQ318442.1	IQ1	Milk
2	C. Coli	OQ318444.1	IQ2	Milk
3	C. Coli	OQ423040.1	IQ13	Milk
4	C. coli	OQ330748.1	IQ15	Human
5	C. coli	OQ338161.1	IQ19	Human

The analysis of the 10 isolates, showed matching 8 (80%) sequences as a *C.* and 2 (20%) of strains as a *C. lari* among the sequenced isolates in table (4).

The 10 Campylobacter strain isolates used in this study's bioinformatics analysis displayed very high similarity (approximately 99%). It is common practice to identify between different Campylobacter spp. Using 16S rRNA gene sequences [15]. Our findings supported the strategy stated and suggested by [16]. The findings did concur with [17] Campylobacter spp. Isolates previously found in Europe and America [18].

Type by Sequence Technique This method of high-resolution bacterial genotyping has been helpful in molecular research. [19] The discovery of two strains that produce gastroenteritis with remarkable efficiency Due to the varied phenotypic, polymorphism, and other contextual factors, it was discovered and recorded in the Global Gene Bank that traditional methods are frequently insufficient. It was diagnosed using the polymerase chain reaction (PCR) method and primers created for molecular diagnosis [17]**.**

According to the registration information and the analysis of the affinity and similarity between the registered bacteria, the results indicated that this bacterium had been registered in the global gene bank. Traditional diagnostics and genetic analysis are crucial for classifying microorganisms. Due to the SSU region's ease of amplification and wide range of variability, even in closely related species, it has been frequently employed in categorization and molecular diagnosis [17].

DNA sequencing was done using genetic analysis software and the NCBI-BLAST program to guarantee the nucleotide sequence before being compared to other worldwide strains. This comparison produced accurate results. An analysis tool called Molecular Evolutionary (BLAST) compares similar gene sequences, evolutionary relationships, and the trajectory of DNA and protein evolution [20].

Due to the potential for transmission through the import and export of different foods and goods and the potential for transmission through infected individuals, this bacterium has expanded widely in various parts of the world [21].

Determination of the sequence of nitrogenous bases, bioinformatics analysis, and the genetic tree Phylogen

The results of the nucleotide sequence analysis of the doubled DNA bundles were shown by the National Center for Biotechnology Information (NCBI) initiative and compared to the available data. The ten transmitted isolates belong to each of them.

The isolate bearing the number OQ318444.1 and registered in the Genome Bank belongs to the bacterium ***Campylobacter coli*** from milk, where the matching percentage with international isolates was 100%, as shown in Figure No (5).

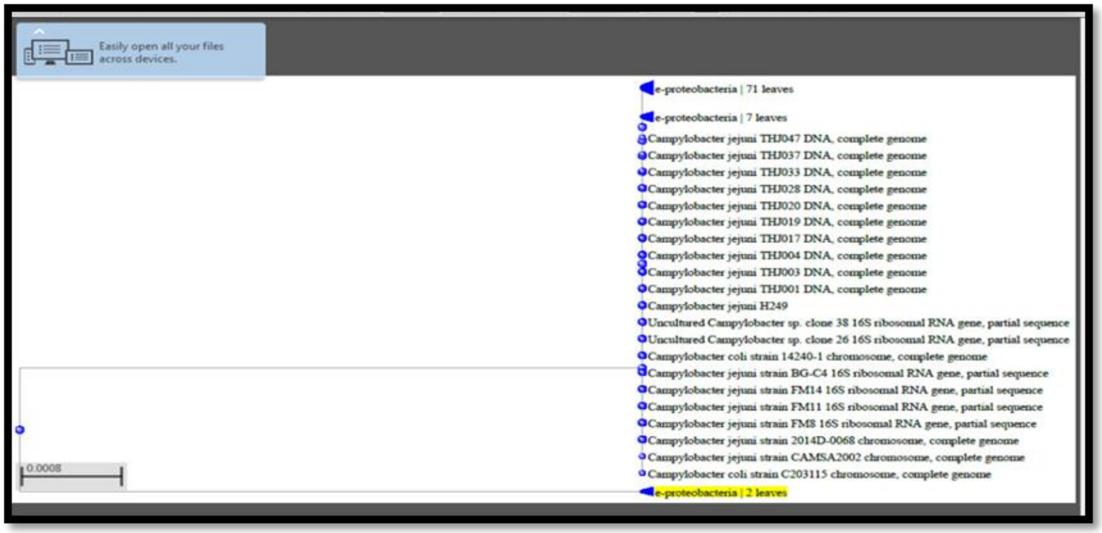

Figure (5) The genetic tree of campylobacter coli (marked in yellow), which was built based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method.

The isolate bearing the number **OQ318442.1** and registered in the Genome Bank belongs to the bacterium *campylobacter lari*, from milk, where the matching percentage with international isolates was 100%, as shown in Figure No (6)

e-proteobacteria | 75 leaves

Campylobacter jejuni strain FM11 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM10 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM9 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM8 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM7 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM6 16S ribosomal RNA gene, partial sequence
Campylobacter coli strain FM5 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM4 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM3 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain FM1 16S ribosomal RNA gene, partial sequence
Campylobacter coli strain P4581 chromosome, complete genome
Campylobacter coli strain C203115 chromosome, complete genome
Campylobacter coli strain C203112 chromosome, complete genome
Campylobacter coli strain C203110 chromosome, complete genome
Campylobacter coli strain C203018 chromosome, complete genome
Campylobacter coli strain C203016 chromosome, complete genome
Campylobacter coli strain FDAARGOS_1465 chromosome, complete genome
Campylobacter jejuni subsp. jejuni strain GB19 chromosome, complete genome
Campylobacter jejuni strain CAMSA2002 chromosome, complete genome
Campylobacter jejuni strain CAMSA2038 chromosome, complete genome
Uncultured Campylobacter sp. clone EPI004 16S ribosomal RNA gene, partial sequence
Campylobacter sp. strain CVASU16 16S ribosomal RNA gene, partial sequence
Campylobacter sp. strain CVASU15 16S ribosomal RNA gene, partial sequence
Campylobacter sp. strain CVASU14 16S ribosomal RNA gene, partial sequence
Campylobacter jejuni strain 2017D-0132 chromosome, complete genome
Campylobacter lari strain IQ1 16S ribosomal RNA gene, partial sequence

Figure (6) The genetic tree of campylobacter lari(marked in yellow), which was built based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method

4. Evaluation of antibiotic susceptibility test.

All ten isolates were subjected to 12 antimicrobial drug tests to determine which were sensitive and which were resistant., as in Figure (7), Which showed significant differences ($p<0.01$). The current result shows high resistance to imipenem is 10(100%) and gentamicin 9(90%), as in Figure 7.

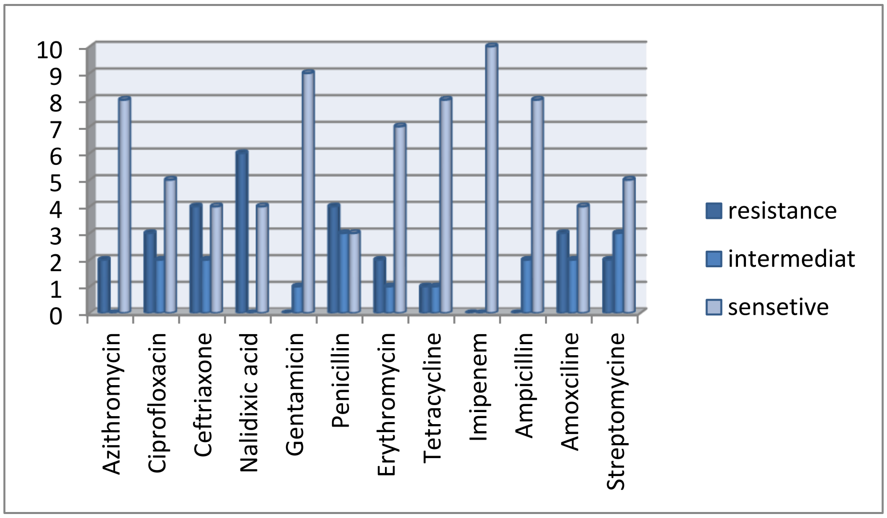

Figure (7): effect of antibiotic with campylobacter coli& campylobacter lari

Numerous nations' isolates of Campylobacter exhibit a wide range of antibiotic resistance patterns, which have been recorded in published research. Strains that thrive in various ecological niches, geographic regions, the usage of antibiotics, and The horizontal gene transfer of resistance determinants is the common factor influencing all of these variations in resistance phenotypes[1].

Discussion

1. Cultural Characteristics:

The samples utilized in this study were cultivated on Campylobacter agar base, an enriched and selective medium that also contained 5% blood and a Campylobacter selective supplement made up of sodium pyruvate, cefoperazone, vancomycin, and cycloheximide. In a sealed jar employing gas packs, the plates were incubated at 42°C for 48 hours under microaerobic conditions (about 80%-90% N2, 5%-10% CO2, 5%-10% O2). [11]

All Campylobacter spp. Colonies are small, mucous, typically greyish to creamy grey, slightly elevated, moist, and frequently create distinct colonies, flat with uneven borders, and non-hemolytic, as illustrated in Figure (1) from the culture of Campylobacter spp. Isolates on selective media. The findings concurred with those of [22-24]; [6] and [1]**.**

2.Prevalence of Campylobacter spp. in milk and milk product

According to the European Centers for Disease Control and Prevention (ECDC) and the Global Enteric Multicenter Study (GEMS), Campylobacter is a significant gastrointestinal pathogen responsible for outbreaks worldwide. Six (6%) positive findings were dispersed among milk and milk products in the current investigation. These results align with a previous investigation in Pakistan, which found that butter and raw milk had the highest levels of Campylobacter.[25]. Additionally, Italy found similar prevalence rates ranging from [26]**,** Tanzania [27], and Erbil, Iraq [1]**.** However, other Iranian research also revealed lower rates. [28], Hatay_Turkey [29]Egypt (4.44%) [30], and India (2.9%) [16].

These variances can be related to, among other things, regional differences, the sensitivity of the detection method, levels of hygiene, eating habits, and the presence of natural Campylobacter reservoirs. [31]. Regarding *Campylobacter* spp. Isolated in this study.

3.Prevalence of Campylobacter spp. According to the Months:

The current investigation aimed to find out how common campylobacter SPP infections were. According to the study's months, and the results are shown in (figure 2), there was a significant difference, p0.05, with February 2 having the lowest prevalence rate of isolation (1%) and October 11 having the greatest (5%). Early spring and early summer had the highest recorded occurrence of Campylobacter Winter has the lowest rate as far as time-dependent prevailing. These results are in accordance with a study from Nigeria that discovered that the incidence of campylobacteriosis increases in the summer and decreases throughout the winter [32] .

Numerous research had linked warm seasons to increased Campylobacter prevalence. [33]Germany [34][35], Egypt [36], Lebanon [37]and Iraq [11]. Although the fundamental cause of this seasonality is yet unknown, it may suggest a potential relationship between temperature and Campylobacter survival and infection transmission [1].

The prevalence of Peak risk for campylobacteriosis occurs in the summer in temperate locations, whereas there is a far less pronounced seasonal pattern in tropical areas [38].

4.prevalence of Campylobacter spp. in Human Stool Samples.

This study appeared that Four (8%) of the 50 stool samples produced Campylobacter species when cultured. Given that the incidence rate of Campylobacter spp. Infections are highest in children under the age of five and decline with age; table (2) demonstrates a significant ($p \leq 0.05$) relationship between age and infection; this result agrees with [39], who identified Campylobacter spp. from stool samples by PCR assay, while much higher than that found by [40], who demonstrated that PCR was more sensitive (100%) than the direct bacterial culture (49%) and who discovered *Campylobacter spp.* in patients with diarrhoea (16.6%) by direct Real-Time PCR by hipo gene primers. The difference in detection rate between this study and other research may be influenced by a variety of variables, including age, season, region, and human immune status. [41]

The study by AL-Hamadani and Saleh (2011) in the Al Diwanyiah governorate indicated that the percentage of *C.coli* and *C.lari* was 8% in children with positive *Campylobacter spp*. Culture, which was consistent with the general recovery rate of *campylobacter ssp.* 5.4% of children with diarrhoea were reported to have an isolated *Campylobacter* species in Turkey [42], Seven per cent in India [43] and 11.1% in Lebanon [44]**.** According to WHO and FAO reports from 2012, Europe and North America had a 9.3 per cent incidence of campylobacteriosis. One of the four main global causes of diarrheal illnesses is *campylobacter*. It is thought to be the most typical bacterial cause of gastroenteritis in people worldwide [45].

Campylobacter's self-limitation and the fastidious nature of the bacteria may be to blame for the low recovery rate by culture method. Other factors that make it difficult to culture the organism include contamination, intestinal flora, loss of viability during transportation, and consumption of proton pump inhibitors (PPI), which have an indirect antibacterial effect [46]. All these may be responsible for a negative predictive value associated with culture of *campylobacter*[11, 46-48].

5. Distribution of Campylobacter isolates according to age

Due to their immature immune systems and the fact that campylobacter infection is regarded as a self-limited disease dependent on the immune system, the results of this study and others like it demonstrated that the most effective age with *Campylobacter* is under five years[49,50]..

These findings agree with that found by other researchers in some points in Brazil, [51] demonstrating a strong correlation between the presence of Campylobacter spp. in infants and young children (0–12 months). In Québec /Canada, the incidence rate in children under four years was significantly higher [52]. Furthermore, [53] revealed that campylobacter infections are particularly common and can occasionally be fatal in children under two. In impoverished countries where Campylobacter is common, infection is typically limited to children, and the illness/infection ratios decline with age, suggesting that early exposure may result in establishing a protective immunity. [54-56].

Molecular identification

Molecular identification by PCR using the 16S rRNA gene:

The availability of a trustworthy species isolation strategy gives All strains data that can be compared across laboratories and over time [48].

Typing by Sequence Method

Sequence Typing of Partial *16S rRNA* Gene from *Campylobacter spp.*

The 10 *Campylobacter* strain isolates used in this study's bioinformatics analysis displayed very high similarity (approximately 99%) with *Campylobacter spp. isolates* previously found in Europe and America [18].

It is common practice to identify between different *Campylobacter spp.* Using 16S rRNA gene sequences [15]. Our findings provided significant evidence favoring the approach mentioned and recommended by [16]. The findings concur with [17] Typing by Sequence Method; It is a high-resolution bacterial genotyping technique that has been useful in molecular studies [19].

The identification of two isolates with high efficiency in the production of gasteroenitierites was recorded in the Global Gene Bank, and it was found that the use of traditional methods is insufficient in most cases due to the heterogeneous phenotype and polymorphism as well as the different environmental conditions. By using primers created for molecular diagnostics, it was determined using the polymerase chain reaction (PCR) technique. [17]**.**

The results showed that the registration of this bacteria was recorded in the global gene bank, as shown in the registration information and the study of the affinity and similarity between the registered bacteria. Traditional diagnosis and genotype determination are essential in the classification of bacteria. The SSU region has been widely used in classification and molecular diagnosis due to its ease of amplification and wide range of variability, even in highly related species [57].

DNA sequencing was to ensure the sequence of nucleotides and then compare it with other international strains. The NCBI-BLAST program was used and gave accurate results by comparing it with international strains, and the genetic analysis program was used. Molecular Evolutionary (BLAST) is an analysis application designed to compare similar gene sequences, evolutionary relationships, and the pattern of DNA and protein evolution [20].

The wide spread of this bacteria in different places around the world is due to the possibility of its transmission through the import and export of various foodstuffs and goods, as well as the possibility of its transmission through people who carry it [21].

Evaluation of antibiotic susceptibility test.

Campylobacter Coli & *Campylobacter Lari* in our study showed high sensitivity to Imipenem 20(83%), gentamycin 12(50%) and ciprofloxacin 13(54%) While 100% resistance to tetracycline, ampicillin, and streptomycin was noted (Figure 8). In published research from various nations throughout the world, the pattern of antibiotic resistance of Campylobacter isolates varies greatly [58].

The current study's finding of ciprofloxacin sensitivity agreed with levels previously reported in Iran (30.77% and 34.4%) [59] [60]**;** [61]. As well as higher than that reported in India [16]**.** The high sensitivity to ampicillin, erythromycin, and gentamicin found in this investigation also agrees with the high sensitivity to gentamicin and streptomycin revealed in a recent Tanzanian study of cattle corpses and raw milk samples [27].

Strains prospering in various ecological niches, geographic regions, the usage of antibiotics, and These variations in resistance phenotypes are all influenced by horizontal gene transfer of resistance determinants. [62-63]**.**

Conclusions:

This study is the first to report prevalence and estimate the effects of some risk factors for *Campylobacter* infection in humans, milk, and milk products. Molecular testing was used to confirm the diagnosis, and it showed that PCR was the fastest, most accurate way to identify the pathogen. Two *Campylobacter* species (*C. et al. lari*) were identified based on the sequence registration of local isolates. The current statistics indicate that the prevalence rate increased in October and November. The infection rates increased with age and were higher when people were younger. To reduce the risks to public health posed by the spread of infections with multiple drug resistance, random antibiotic use should be regulated according to the results of the antibiotic susceptibility test performed to examine *campylobacter* resistance to antibiotics.

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