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Isolation and identification of *Campylobacter spp.* from human, raw milk and milk product by using molecular technique in kerbala governorate

Athesis

Submitted to the council of the College of Veterinary Medicine at University of Kerbala as a Partial fulfillment of the Requirement for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary Public Health

by

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بسم الله الرَّحْمنِ الرَّحِيمِ

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Dedication

I dedicate the fruit of my effort for Allah's sake

To " Imam AL-Hussein and Imam Al-Abbas "(peace be upon him)

The one gave me the strength and support

"My father"

To whom her prayer was the secret of my success

"My beloved mother"

To My husband, "Sarmad," , whose patience and encouragement enabled me to finish this work

To whom mother's soul and her live gaiety

"My daughters(Afnan and Jumana)"

And to those who supported me spiritually and morally,

″ My sisters"

To whom stood I with

"My friends"

&

For Any person who puts a word of knowledge into this thesis.

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List of Abbreviation

	Abbreviation Meaning
16S rRNA	**16 Svedberg Ribosomal Ribonucleic Acid**
AMR	**Antimicrobial resistance**
API	**Application Programming Interface**
ARB	**Antibiotic resistant bacteria**
ARG_S	**antibiotic-resistant genes**

BHI	**brain heart infusion broth**
C	***Campylobacter***
CFF	***Campylobacter fetus* subsp *fetus***
CFV	***C. fetus* subsp. *Venerealis***
CLSI	**Clinical & laboratory standard institute**
E. coli	***Escherichia Coli***
EU	**European Union (EU)**
FAO	**Food and Agriculture Organization**
FDA	**Food and Drug Administration**
FERG	**Foodborne Disease Burden Epidemiology Reference Group**
GBD	**Guillain-Barre disease**
HACCP	**Hazard Analysis and Critical Control Point**
HAS	**heat-soluble stable antigens**
HIV	**human immunodeficiency virus**
HLA-B27	**histocompatibility antigen**
IBS	**inflammatory bowel syndrome**
IBS	**irritable bowel syndrome**
ISO	**International Organization for Standardization**
MCCDA	**modified cefoperazone deoxycholate agar**
MFD	**Miller Fisher disease**
MIC	**Minimal inhibitory concentration**
MLEE	**Multi-Locus Enzyme Electrophoresis**
MLST	**Multilocus sequence typing**
MRD	**maximum recovery diluent**

NARMS	**National Antimicrobial Resistance Monitoring System**
NCBI	**National Center For Biotechnology Information**
PCR	**Polymerase chain reaction**
PFGE	**Pulsed Field Gel Electrophoresis**
PI-IBS	**Post-infection irritable bowel syndrome**
ReA	**Reactive arthritis**
SARS	**severe acute respiratory syndrome**
SPSS	**statistical package for social Sciences**
USA	**United States of America**
UTI	**urinary tract infection**
VWA	**Victorian Workcover Authority**
wgMLST	**GenomeTrakr utilizes Whole-Genome MLST**
WGS	**Whole Genome Sequence**
WHO	**World Health Organization**

Summary:

Campylobacter spp. is a major food born pathogen that is increasingly found worldwide and that is transmitted to human through meat ,raw milk and dairy products.

This study aimed to determine the prevalence of *Campylobacter spp*. in human , raw milk and milk products sample in holly Karbala city, and the detection the antibiotic sensitivity of these isolates , the present study was beginning from October 2022 to the end of March 2023 ,on Two hundred (200) samples were aseptically collected as fallow : 100 sample of raw milk and 100 sample of milk products from different local shops and farms which distributed in kerbala provinces as well as 100 samples from human aged from 2 month to 10 year suffering from diarrhea, fever, and abdominal pain at the General Children's Hospital & Al-Hussein-Teaching-Hospital .

Samples were cultured immediately onto enriched and selective media . that appear small, mucoid colonies with typically grayish coloration and some have to be creamy grey in color, slightly raised, moist, and frequently produce discrete colonies, flat with irregular edges, and non-hemolytic at 24-48 hours were the characteristics of *Campylobacter spp*. colonies isolated on Campylobacter agar base selective media.

By using the polymerase chain reaction method, 38 isolates out of 300 samples (12.6%) were confirmed to be *Campylobacter spp.* . From results that showed *Campylobacter spp.* sixteen isolates were subjected for sequencing to detect the species of *Campylobacter* there was recorded as four species (*C. jejuni, C.coli ,C.lari & C.upsaliense*).

According to antimicrobials susceptibility through disk diffusion assay ,Antimicrobial susceptibility testing for *C.Jejuni & C.upsaliense* showed complete sensitivity to ciprofloxacin , gentamycin, and Imipenem while total resistance to tetracycline ,streptomycin & cephalexin acid . On the other hand, *C.Coli & C.lari* total sensitive to gentamycin ,imipenem and azithromycin while resistance to ampicillin & amoxicillin was also observed.

This *Campylobacter spp.* resistance pattern discovered in this study is quite concerning due to the insusceptibility to the previously mentioned antibiotics, which are routinely employed as the medications of choice for campylobacteriosis treatment. The warm season was associated with an increase in *Campylobacter* prevalence in raw milk. Because of the possible public health risks, these levels of prevalence and resistance merit additional investigation and appropriate remedies.

Chapter One

Introduction

Introduction :

The gram-negative, tiny, S-shaped or curve bacterial infection known as *campylobacter* is zoonotic. It is widely acknowledged the majority widely *Campylobacter spp.* Is assumed to be mainly a foodborne disease , also the importance of milk as source of human Campylobacter enteritis was confirmed to the world , as of its The genus Campylobacter has received preliminary taxonomic confirmation expanded to include a number of important diseases that affect both humans and animals. Over the past few decades, It has repeatedly been identified as the pathogen in outbreaks in both developed and poor countries (Almashhadany, 2021)**.**

Campylobacter bacteria are ubiquitous in cattle and dairy farms. Poorly cleaned machines, bovine diseases (mastitis), and, most often, fecal contamination of the milk from the known reservoir represent documented causes of milk pollution during the reported outbreaks of *Campylobacter* infection , *Campylobacter* infections are most commonly caused by ingesting contaminated food or drink , The presence of *Campylobacter* in raw milk or dairy products frequently implies the danger of zoonotic transmission to humans (Bolton, 2015)**.**

Milk is a basic food in human diet either in its original form or in a various dairy products, as it contains high quality of animal protein and fats as well as vitamins and minerals which are important nutrients either for young, adult or elderly people. On the other hand, milk has a high water activity (aw=0.99) and slight acidic (El-Kholy *et al.,* 2016). As a result, milk is a good substrate for microbe development, and raw milk serves as the primary source of different infections such as Campylobacter (Leedom, 2006).

Campylobacter is the primary cause of zoonotic illnesses in many countries, and the public health burden of campylobacteriosis is growing on a daily basis. (Horrocks *et al*., 2009)**.**

The epidemiology of *Campylobacter* infection in affluent countries differs dramatically from that in developing countries. *Campylobacter* enteritis has no preference for season in impoverished nations, but campylobacteriosis epidemics occur in developed countries throughout the summer and autumn (Almashhadany, 2021; Platts-Mills & Kosek, 2014)

A variety of transmission mechanisms have been blamed for the transfer of *Campylobacter* spp. to humans, including raw or underdone poultry or meat, raw milk, and milk products (Hussain *et al.*, 2007)**.**

Dairy products have been identified as the primary cause of *Campylobacter* infection in humans, ranking top among foods linked to Campylobacteriosis outbreaks (Painter *et al.*, 2013)(Taylor *et al*., 2013)

Campylobacteriosis, a zoonotic infection, is the most common result of *Campylobacter* species exposure through food intake, which is often diagnosed as gastroenteritis (Coker *et al.*, 2002) It has been shown that Campylobacter species can cling to and attack human intestinal epithelial cells via their flagellum, causing intestinal barrier damage, producing toxins, and avoiding immune responses (Asmat & Khan, 2020) . Symptoms include abdominal pains, diarrhoea (usually bloody), vomiting, headache, nausea, dizziness, lethargy, emesis, and fever (Khademi *et al*., 2021)

Most campylobacteriosis side effects are minor or only require a few days of hospitalization. But in extreme cases, campylobacteriosis can lead to post-infection complications like septicemia, reactive arthritis, inflammatory

bowel syndrome (IBS), Guillain-Barre disease (GBD), Miller Fisher disease (MFD), a peripheral demyelinating polyneuropathy that causes weakness or momentary paralysis, or even death, particularly in young children, the elderly, and immune-compromised individuals (Hussain *et al.*, 2007)**.**

Along with the burden of illnesses brought on by these bacterial pathogens, the emergence of antibiotic-resistant *Campylobacter* strains is another cost on public health, one that may be worse in developing nations where the use of antibiotics is generally unregulated. (Rousham *et al.*, 2018)(Omulo *et al.*, 2017)**.**

Due to the fast appearance and dissemination of antibiotic-resistant bacteria and genes on a worldwide scale, antibiotic resistance is recognized as a One Health issue (Rousham *et al.*, 2018). Animal feces, human waste, and wastewater effluents are the main sources of antibiotic resistant bacteria (ARB), which can spread and cause antibiotic-resistant genes (ARGs) to evolve in exposed bacteria ,antibiotic resistance can develop as a result of vertical gene transfer or genetic interactions between and within bacterial species (Holmes *et al.*, 2016).

Aquatic ecosystems are regarded as one of the main reservoirs of ARB and ARGs, are emerging environmental contaminants (Adiguzel *et al*., 2018)

Objectives of the study:

At the level of the Kerbala governorate, this study is the first to examine the isolation and identification of *Campylobacter spp.* from human , raw milk and milk products by :

1.Utilizing culture to isolate and identify the bacteria before conducting a molecular analysis for confirmation .

2.Examine the purity (sequence) to identify type of this bacteria.

3. conduct antibiotic susceptibility test to detect the resistance & sensitivity of the bacteria to the antibiotics.

Chapter Two

Literatures Review

2.1 History of *Campylobacter spp.*

In 1886 the first *Campylobacter* was isolated by Theodor Escherich from colon of a child suffering from diarrhea and died of "cholera infantum" later. The bacterium was spiral in shape and was non cultural (Johnson, 1986). The organism was identified as Vibrio on the basis of resemblance in morphological characteristics (Epps *et al*., 2013). Later this bacterium was isolated in huge numbers from uterine mucus of pregnant sheep by two veterinarians in Britain, from bovine and ovine in 1913 by McFadyean and Stockman (Skirrow, 2006) . In 1927, Vibrio like organism was isolated from fecal samples of bovine and was named as Vibrio jejuni (*Campylobacter* fetus nowadays). In United States *Campylobacters* were isolated from cattle in 1931, from humans in 1938 and from pigs in 1940. *Campylobacter spp.* were differentiated from Vibrio for the first time by King in 1950 by culturing the bacteria at high temperature. A new genus was proposed on the basis of no fermentative metabolism, low DNA base composition and microaerophilic condition for growth by Sebald and Veron in 1963 as *Campylobacter* (On 2001). After 1970s *Campylobacter* attracted the interests of scientists for research in humans and animals (Butzler, 2004). Currently *Campylobacter* genus is composed of 17 species and 6 subspecies (On 2001) and is the second most important diarrhea causing bacteria in developed countries of the world.

2.2 General Characteristics of *Campylobacter:*

Campylobacter species are small, motile and gram-negative bacteria. *Campylobacter* are 0.5-5 μm long and 0.2-0.8 μm wide. They are S shaped or

comma shaped small rods, oxidase and catalase positive bacteria and sometimes found in coccoid form (when exposed to oxygen). On exposure to unfavorable condition they are found in coccoid form, usually viable bacteria, but not culturable cells (VNBC). Coccoid *Campylobacters* are mostly found in cultures that are exposed to oxygen and older (Vandamme & On, 2001).They are motile bacteria and "cork screw" motion is the unique characteristics of *Campylobacter* due to presence of flagella on one end (unipolar) and on both sides (bipolar) of the pathogenic organism (Humphrey *et al*., 2007).They are microaerophilic in nature and require low level of oxygen and nitrogen and high level of carbon dioxide present in normal environment. Microaerobic condition for *Campylobacter* consists of 5% O2, 10% CO2 and 85% Nitrogen for optimal growth. Most of the species of this genus are thermo-tolerant and can grow in high range of temperatures from 37˚C to 46˚C. These organisms have an optimal temperature of 42˚C but cannot grow below 30˚C and above 50˚C (Stephan. L. W. On *et al.*, 2014). They are sensitive to light, low pH, salt and reduce ability of multiplication by exposing to host's external environment and freezing, processing, and storage (Franzoi *et al.*, 2022).

2.3 Epidemiology

2.3.1. Prevalence

The estimation of accurate prevalence of campylobacteriosis in human is difficult due to under reporting enteric disease causes by other organisms. In the UK, 8.7/1000 person suffered annually from this infection. In US during the last decades of 20th century it was found that about 8% of the retail

meat was contaminated with *Campylobacter* (Lu *et al.*, 2018) . During 2007 to 2009, chicken broiler flock were studied and 54% of the flocks were identified positive for *Campylobacter*. The contribution of *C. coli* was 30% and that of *C. jejuni* was 17% (Bertasi *et al.*, 2016)Campylobacter species were present (18.9%) on meat carcasses in Brazil in 2010. *Campylobacter jejuni* was highly prevalent as compared to *Campylobacter coli* in meat supply chain in Santa Fe region in Argentina. Out of 152 *Campylobacter*, 105 were confirmed as *Campylobacter jejuni*, 43 as *Campylobacter coli*, and 4 as other species (Rossler *et al.*, 2019)Prevalence of *Campylobacter* was 71.2% and 75.8% in live poultry and carcasses in European Union. In Estonia it ranged from 2% to 100% and 50% in Hungary (Sandberg *et al.*, 2015). In Czech republic during 2007, *Campylobacter* species were present in 75% of chilled poultry meat and reduced to 37% in frozen (Bardoň *et al*., 2011)The prevalence of *C. jejuni* was 64% in France in 2007 in chicken meat sold from retail outlets (Saint-Cyr *et al.*, 2017). In Greece, *C. coli* was most abundantly isolated from *C. jejuni* from carcasses of small ruminants from 2007 to 2009 with 76.2% and 21.4%, respectively (Lazou *et al*., 2014).During 2009 to 2010, the overall prevalence of *Campylobacter* was 83% after packaging and gradually reduced after processing steps of scalding and chilling in Australia (Duffy *et al*., 2018)

In New Zealand, *Campylobacter jejuni* bacteriophage was isolated from 28% of the chicken rinse polled samples from 37 flocks (Owens *et al.*, 2013)In Pakistan during 2002-2006, the prevalence in poultry was 48%, in raw beef 10.9%, and in mutton was 5.1%. In other foods, it was 32% in sandwiches, 11% in cheese, and 10.2% in raw milk (Miljković-Selimović *et*

al., 2021)Two hundred and seventy-five (275) *Campylobacter species* were identified in 767 meat samples collected from slaughterhouses in Shandong province of china during 2008. The number of *C. jejuni* positive were 208, *C. coli* were 53, and 14 were unidentified (Miljković-Selimović *et al*., 2021). The prevalence of *Campylobacter* species in fresh chicken meat and byproducts was 64% on retail level in Sapporo, Japan in 2006 (Bertasi *et al.*, 2016). The range of prevalence of *Campylobacter* in duck was 0% to 84% from different sources in Penang Malaysia during 2009 to 2011 (Heckman, 2019). In Iran, prevalence of *Campylobacter* was 36.5% in 2004 (Stephan . L. W. On *et al*., 2014), 29% in retail beef and chicken in 2006 and 2007 (Loss *et al*., 2015) and 62.1% in turkeys in 2007 (Rahimi, Sepehri, *et al*., 2013)

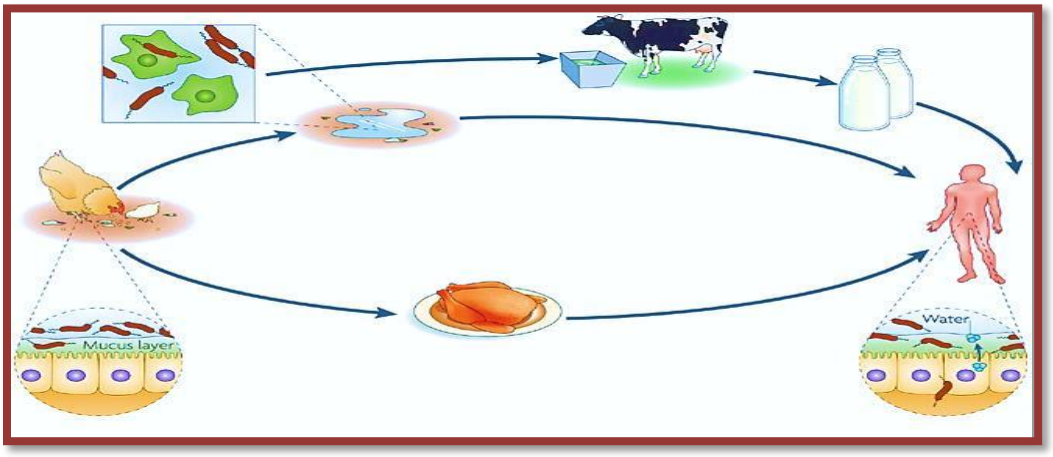
2.3.2. Sources

Campylobacteriosis is well known foodborne disease caused by *Campylobacter species* by contacting with contaminated water and food. Most of cases occurred due to eating of semi cooked poultry meat and product; raw vegetables and salads, unpasteurized milk and untreated water from common environmental sources (Karlyshev *et al*., 2005) Thermo tolerant *Campylobacter* species are important zoonotic pathogens and commonly isolated from a verity of animal species i.e., poultry, sheep, cattle, rodents, wild birds and pets (Stephan. L. W. On et al., 2014).Poultry is considered a primary source of *Campylobacter*, and the prevalence in birds is more than other animal species due to its high body temperature (Upadhyay *et al.*, 2019) All birds, including commercial, wild and backyard poultry can colonized with these pathogens without showing any clinical signs and symptoms. *Campylobacter jejuni* and *Campylobacter coli* are frequently

present in normal intestine of all warm blooded animals while *C. upsaliensis* and *C. helveticus* in dogs and humans are the only reservoir host for *C. showae, C. rectus, C. curvus, and C. concisus* (Sommerlad & Hendrixson, 2007)

2.3.3. Transmission

Three main routes for transmission of *Campylobacter species* are food, water, and direct contact with reservoir or infected host (Barakat *et al.*, 2015) Consumption of uncooked poultry and red meat, unpasteurized milk, contaminated water, cross contamination from raw meat to vegetables and direct contact with animals are known to be potential sources for transmission as in figure (2_3) (Olivier *et al.*, 2021) .*Campylobacter* are normal inhabitants and present in the intestinal tract of all worm blooded animals and chances of cross contamination is more at the time of slaughtering from intestinal content to the meat surface. Poultry birds are the primary source of campylobacteriosis and is indicated as a significant risk factor in many case control studies (Damborg *et al*., 2004 and Kipper *et al.*, 2019). Evidence for human to human transmission is obtained in case of outbreak and thought to be source of infection in 95% cases. Foreign travelling to developing world, swimming and companion animals can be an important source for human campylobacteriosis (Ursing *et al.*, 1994 ;Albert J. Lastovica1 . Stephen L. W. On Zhang, 2014 and Stephen L. W,* On *et al*., 2017)



Figure(2-1): The Source of *Campylobacter spp.* Infection, Human can be Infected by Ingestion Contaminated Water, Milk and Poultry Meat(Twenge *et al*., 2010)

2.3.4. Risk factors

Any disease causation and transmission needs to fulfill the phenomena of epidemiological triangle. The transmission chain consists of host agent and environment. Some are the factors enhances the disease and declines the host's defense systems. The main risk factors for *Campylobacter* infection in humans are: consumption of contaminated food, raw meat (Hull *et al.*, 2021)untreated water, raw milk, sausages, barbeques, semi cooked ham burgers; handling of poultry birds and other animals, slaughtering and processing of animals and birds (Tee & Mijch, 1998; Rushton & Irwing, 2009& Stephan . L. W. On et al., 2017)

Travelling to other countries and swimming is associated with campylobacteriosis (Vandamme & On, 2001).The incidence of campylobacterioisis in humans are greater in warmer months in most of the developed countries (Adiguzel *et al*., 2018). Poultry colonization is attributed to: lack of hygienic practices (Bolton, 2015) depopulation of flock in several

batches, presence of other farm animals and pets (Smith, 2016), presence of multiple poultry houses, higher age of broiler at slaughtering time, existence of rodents at farm (Bardoň *et al*., 2011), use of nipples drinkers (Ge *et al.*, 2013) huge size of flock (Konkel *et al.*, 2004)receiving chicks from individual hatchery (Mitchell *et al.*, 2013) increasing ventilation during summer (Kashoma *et al*., 2016) and lack of flies screen (Elmalı & Can, 2019).

2.4 Food -borne illness:

Foodborne infections are a serious public health issue around the world. Most of the cases are due to consumption and contamination of food from animal origin. In developing countries the problems are neglected and not frequently reported. Campylobacter is an important foodborne pathogen. This organism is not frequently reported in Iraq and no information on prevalence and antibiotic resistance is available.

Zoonosis, or diseases of animal origin, are illnesses that spread from animals to humans through direct contact, indirect environmental contact, or through consumption of contaminated food (Authority, 2017)**.**

The importance of animal health and hygiene for the production of safe and wholesome meat and milk was first discussed by doctor Silvio J. Bonansea in his paper titled "Veterinary Hygiene Applied to the Protection of Man against Zoonosis" in 1906.The first meeting of the Expert Group on Zoonosis, which was established by the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) in November 1950 in Geneva, resulted in the identification of 86 diseases that are transmitted from animals to humans. This was almost 50 years later. 20 of

those illnesses were brought on by bacteria.800 of the 1400 pathogens thought to cause human diseases today are thought to be animal-derived. **(Chlebicz&Śliżewska,2018) .**

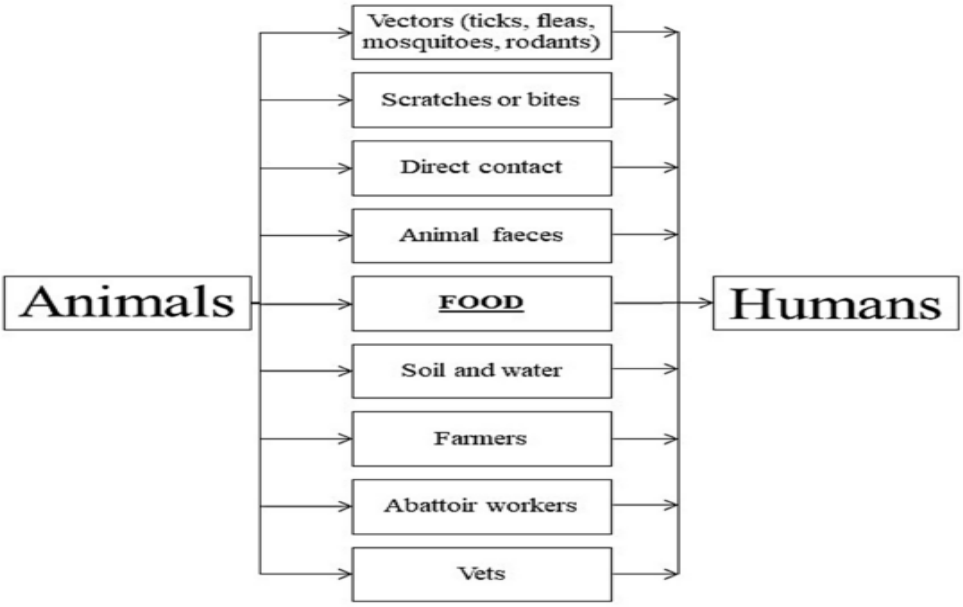


Figure (2- 2): Food borne illness (Chlebicz & Śliżewska, 2018)

Many foodborne illnesses, including *Campylobacter* infections, have been linked to raw (unpasteurized) milk. Campylobacteriosis cases caused by consumption of unpasteurized milk have been reported all over the world. (Resistance, 2019)

Throughout history, mandatory pasteurization and raw milk sales regulations are associated with a decreased of milk-borne disease outbreaks. Before the 1950s, about a quarter of all foodborne infections were attributed to milk (Dewey-Mattia *et al*., 2018)**.**Following the introduction of regulations recommending milk pasteurization, milk was involved with less than one percent of reported foodborne disease outbreaks (Dewey-Mattia *et al*., 2018)

However, as more nations have legalized the sale of raw milk, this number appears to be rising recently. For instance, from 2007 through 2012, the average number of outbreaks in the USA linked to raw milk was four times higher than it was from 1993 through 2006(Mylius *et al*., n.d.). Additionally, the proportion of outbreaks associated with raw milk increased from 2% to 5% from 2007 to 2009 through 2010–2012 (Mylius *et al*., n.d.)**.**

Several pathogens, including *Escherichia coli, Mycobacterium bovis, Listeria monocytogenes, Campylobacter*, *Brucella,* and *Salmonella*, are primarily found in raw milk (Chlebicz & Śliżewska, 2018)

In all of these, *Campylobacter* is the most common zoonotic infection in many nations, and the burden of campylobacteriosis on the public health system is growing daily (Chlebicz & Śliżewska, 2018) , Campylobacteriosis is a digestive disorder that primarily affects children, the elderly, people with underlying medical conditions, and people who are immunocompromised, It typically has a self-limited course, and antibiotic therapy is not typically recommended (Nachamkin *et al*., 2008)

Numerous animal species carry the major cause of diarrhea in the world, campylobacter, which is easily spread through tainted food or water ,the organism can persist in the mammary glands of cows, and raw milk can be a source of transmission. (Leedom, 2006) .

A total of 23 foodborne outbreaks of Campylobacter disease between 1980 and 1982 were reported to the Centers for Disease Control and Prevention. Of these outbreaks, 14 (61%) were linked to consuming raw milk (Leedom, 2006)**.**

It is undeniable that consuming raw milk carries infection risks. Pathogens can infect milk even from cows that are physically healthy (Fagnani *et al.*, 2019). Most recently, a higher risk of hemolytic uremic syndrome was linked to consuming bulk milk purchased directly from the producer. Therefore, we must take into account the possibility that drinking raw milk could be harmful to the general public's health (Fagnani *et al*., 2019)**.**

2.5 Campylobacteriosis

A zoonotic disease that is spread to humans via animals or animal products, campylobacteriosis is a word used to describe infectious disorders caused by Campylobacters. (Organization, 2013).

Campylobacter enteritis, caused by thermo tolerant *Campylobacter species,* is the only type of campylobacteriosis that is significantly important for global public health *C. coli* and *C. jejuni* are the two most common species. (Scallan *et al.*, 2015)

Human campylobacteriosis is primarily brought on by *Campylobacter jejuni*, with minor contributions from *C. coli* and other *Campylobacter species.* Human campylobacteriosis often appears 1–5 days after exposure and is characterized by vomiting for a period of 5-7 days, fever, watery and occasionally bloody diarrhea, and abdominal cramps (Skarp *et al.*, 2016). *Campylobacter* has an incubation period of 3 days, with a range of 18 hours to 8 days (Horn & Lake, 2013)**.**

When an organism enters the body, attaches to, and internalizes in the gut epithelial cells, infection results. Inflammation, watery, frequently bloody diarrhea, dysentery, and extremely painful stomach cramps are possible symptoms. Infections typically go away on their own in 2 to 10 days.

However, in more severe cases, issues with sepsis or arthritis may arise, necessitating the use of antibiotics, notably erythromycin (Smith, 2016) Guillain-Barré syndrome (GBS), a post-infection auto-immune condition marked by an ascending and frequently chronic paralysis, may develop in extreme cases(Smith, 2016).

Regarding how the organism causes Guillain-Barré syndrome, little is known. It is believed to involve, however, molecular mimicry between peripheral nerve cells and microbial antigens. This can lead to the immune system mistakenly identifying its own nerve tissues as an antigen, causing the immune cells to attack and kill the tissues. (Smith, 2016) In the absence of antibiotic therapy, infections are often acute and self-limiting, healing themselves within a week (Taylor *et al*., 2013).

2.6 Complication of human Campylobacteriosis

Campylobacter jejuni complications are rare, locally complications occur due to direct spread of *Campylobacter jejuni* from the gastrointestinal system and include gastrointestinal bleeding, pancreatitis, cholecystitis, and peritonitis (Vandamme & Goossens, 1992)**;**(Butzler, 2004).

Campylobacter jejuni has risk manifestation of developing irritable bowel syndrome (IBS) ,which is a chronic gastrointestinal manifestation occur due to the major effects of bacterial toxins on the intestine which include prolong bowel disorder, inflammation, local tissue damage and slow healing injury (Mungai et al., 2015) and irritable bowel syndrome develops in up to 36% of patients with Campylobacteriosis within 1–2 years after infection (WHO, 2017) **,**Extra intestinal infections include meningitis, osteomylitis,

endocarditis ,neonatal sepsis(Abdulazeez, 2022)**.**Septic abortion, erythema nodosum and hemolytic-uremic syndrome (Petersen *et al*., 2021)**.**

2.7 Pathogenesis

2.7.1 Incubation period

Campylobacter is highly zoonotic and pathogenic bacteria having very low infectious dose, only 500-800 cells are sufficient for causing infection in host. The normal incubation for *Campylobacter* species are 24 to 72 hours after contact with *Campylobacter* by the host with contaminated water or food but it can be exceeded up to 7 days or more depending on the number of cells in inoculums. The cardinal signs and symptoms of Campylobacter infection in humans are diarrhea, abdominal cramps, dehydration, fever, headache, myalgia, and chilling. In humans especially in children diarrhea is common, range from mild to severe and occasionally associated with dysentery (Iovine & Blaser, 2004)The illness is self-limiting and infection remains at peak for two days and then gradually decreases over a week. Mortality is very low in adult humans and high in children and immune-compromised peoples (Zilbauer *et al*., 2008 and Louwen *et al.*, 2012).The complications are Guillain-Barré Syndrome, Reactive Arthritis and Miller Fisher syndrome. GBS is an autoimmune disorder causing neuromuscular paralysis is found very rarely i.e., 1 case per 1000 cases of campylobacteriosis (Koga *et al*., 2005)

2.7.2. Virulence

The process of pathogenesis of *Campylobacter* starts after adhesion of the pathogenic bacteria to intestinal cells, colonization and finally invasion of the cells after ingestion by the host. Flagella, present *Campylobacter*, help in motility and entering into non-phagocytic cells, and are important for pathogenesis in humans and commensalism in other warm-blooded animals. Several proteins, like CadF (outer membrane protein), JlpA (surface exposed lipoprotein), CiaB (secreted protein), CdtA (cytolethal distending toxin), and other proteins help in the process of pathogenesis (Konkel *et al.,* 1999 ;Jin *et al.,* 2001 and Baglie, 2004).

C. jejuni produces capsule (polysaccharide) for the adhesion and invasion process to the epithelial cell of intestine (Parkhill *et al.*, 2000 and Adiguzel *et al*., 2018).

2.7.3. Motility

Campylobacter has one or two polar flagella and helical cell shape. Flagella helps in rotatory cell moment and helical shape helps in corkscrew rotation. The flagella of Campylobacter consist of basal body, hook, and filament. The filament is capped by FliD and other major protein FlaA and minor protein FlaB of the filament help in colonization in both humans and animals (Bolton, 2015) and change in these genes paralyses the bacteria, which then loses the ability of invasion but can still adhere to epithelial (Smith, 2016).Basel body is embedded in the cytoplasm and inner membrane of the cell and composed of distal and proximal subunit, anchor ring and C ring. The hook basal body is comprised of several protein which helps in

attachment of rods to inner membrane (Petersen *et al.,* 2021). The motor component (MotA and MotB), P ring in peptidoglycan (FlgI), L ring outer membrane (FlgH), and minor hook component (FlgE and FlgK) are important component of flagella helping in pathogenesis (Sommerlad & Hendrixson, 2007).

2.7.4. Adhesion

Campylobacter species adhere to epithelial cell of host with the help of adhesions, present on bacterial surface through the process of adhesion (Bolton, 2015). The process of adhesion is carried out by various genes and leads to colonization of bacteria in humans and animals. The cadF gene is highly conserved, encodes fibronectin binding outer membrane protein, which mediates adhesion (Hofreuter *et al.,* 2006)Glycoprotein found in epithelial binds to fibronectin, trigger signaling to activate Rac1 and Cdc42 which induce the process of internalization of *Campylobacter* (Misiewicz *et al.,* n.d.)Another gene, capA encodes *Campylobacter* adhesion protein A is thought to be an auto-transporter lipoprotein helps in adherence and mutation and causes reduction of adhesion and invasion capacity of Campylobacter in humans and poultry (Ashgar *et al.,* 2007). The combination of FlpA and CadF is very essential for the process of adhesion and ultimately invasion of *C. jejuni.* Virulent plasmids (pVir) found in *C. jejuni* contributes in adhesion and mutation in one of the plasmid genes reduces adhesion and invasion significantly (Anjum, 2013).

2.7.5. Invasion

In order to cause infection in host, a pathogen must to invade tissue, withstand or avoid the immune system, avoid disturbance by the gut microflora, and multiply. Invasion is the important step in campylobacteriosis for the disease development. *Campylobacter* have to invade the apical basal polarity and junctional complexes present in healthy intestine of host for disease development. The flagella in *Campylobacter* serve as an export apparatus that secretes non-flagellar proteins during invasion. This systems secretes FlaC and the cia proteins in the cytoplasm of host cell, which are important for the process of colonization and invasion (Petersen *et al.*, 2021). Besides these, CiaC is also considered to be responsible for full invasion and CiaI for intracellular survival (Koolman *et al.*, 2016) Invasion is reduced significantly by mutation in flaA, flab, flaE and ciaB genes in *Campylobacter* (Koolman *et al.*, 2016)

2.7.6. Toxins

Various types of toxins have been produced by *Campylobacter*, but the most important and verified toxin is cytolethal distending toxin (CDT). Three subunits of CDT toxin are CdtA, CdtB and CdtC. The cdt toxins contributes to bloody diarrhea in host by colonization and distension of epithelial cells (Stephan . L. W. On *et al*., 2014). The CdtA and CdtC subunits help in binding of toxin to cell membrane and deliver CdtB, which is enzymatic active sub unit (Abdulazeez, 2022) .

2.7.7. Colonization

Campylobacter species do not produce any clinical signs and lesion in animals and poultry birds. Chicken are the key source of *Campylobacter* and very low dose as 30 to 40 colony forming units are sufficient for colonization. The bacteria colonize rapidly in 24 hours after inoculation and maximum population in cecum is found after 5 days of inoculation (Knudsen *et al.,* 2006). In poultry the bacteria colonize the ceca, resides in the mucus layer over the crypts of the villi in the intestine, and then translocate to internal organs (Rule, 2016). Majority of the birds shed *Campylobacter* species continuously and colonization is slightly declined after 4 weeks of age. *C. jejuni* is most commonly isolated from broiler and its meat products (Smith, 2016).

2.8 Clinical diseases and treatment

2.8.1Clinical diseases and treatment options in humans

Human campylobacteriosis commonly manifests 1-5 days after exposure and is characterized by watery and sometimes bloody diarrhoea, fever, stomach pains, and vomiting that lasts approximately 5-7 days. (Skarp *et al.,* 2016). The incubation period of *Campylobacter* is 3 days with a range of 18 hours to 8 days (Horn & Lake, 2013). In the absence of antibiotic treatment, infections are often acute and self-limiting, clearing within a week .(Taylor *et al.,* 2013)**.** *Campylobacter* infections are generally mild, but can be fatal among very young children, elderly and immunosuppressed

Despite the fact that *Campylobacter jejuni* and *Campylobacter coli* cause the most majority (95%) of clinical illnesses, over 15 different *Campylobacter* species have been found from human infections (Sahin *et al.*, 2017) . In humans, the infective dose of *Campylobacter spp*. can be as low as 500 organisms, and the average incubation period is roughly 3 days (Dai *et al*., 2020).

More than 80% of patients have abdominal pain and diarrhea, while around half have fever, myalgia, and headache, a lesser proportion of individuals (10-15%) experience vomiting and blood in their feces. The beginning of diarrhea, which is usually copious and watery, might be sudden, or it can be preceded by a prodromal period of flu-like symptoms. Typically, the diarrhea stopped within 4-7 days, however some individuals can experience diarrhea for up to 2 weeks. Aside from enter colitis, human extra intestinal symptoms include abscesses, meningitis, and bacteremia (Sproston *et al.*, 2018).These conditions are more happen commonly in immunocompromised, pregnant women and elderly patients (Dai *et al*., 2020)

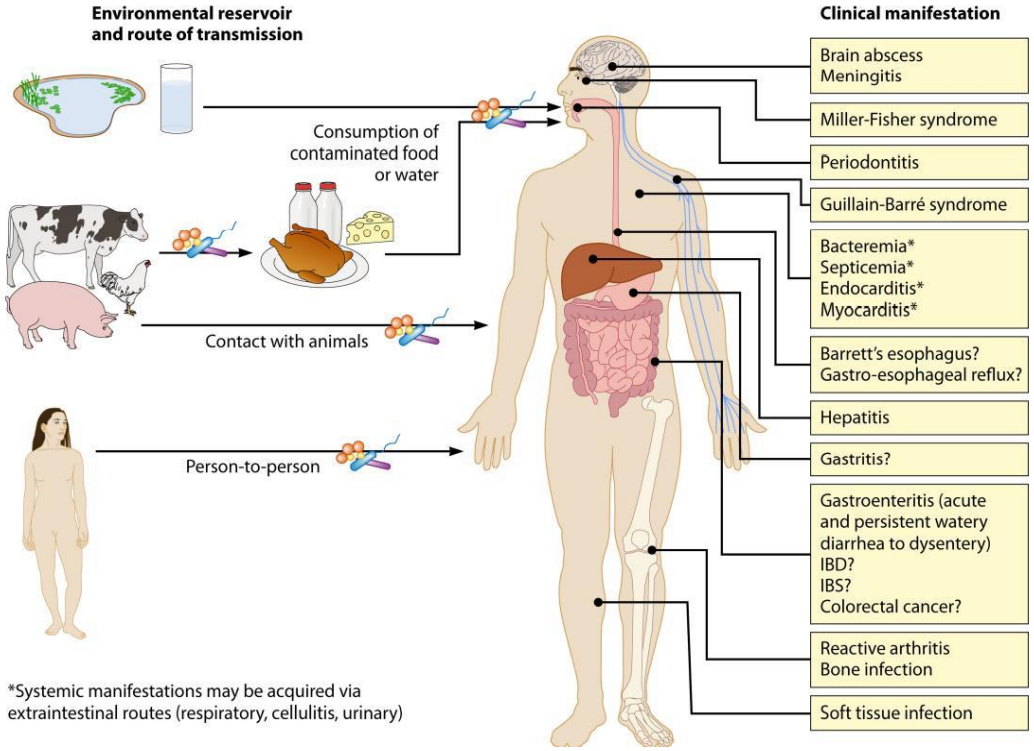
Although the disease is usually self-limiting, antibiotic treatment with a fluoroquinolone or macrolide is becoming more common, with a recent study estimating that up to 80% of people in the community receive an oral antibiotic, most commonly a 3-5 day course of a macrolide antibiotic such as azithromycin. The usage of fluoroquinolone drugs has resulted in resistance, with a 75-90% incidence of fluoroquinolone resistance found in clinical Campylobacter isolates from various countries. As a result, macrolides are

currently the first-line therapy for human campylobacteriosis (Scallan Walter *et al*., 2020)**.**

In addition to acute morbidity, persistent sequelae are frequently described in people after Campylobacter infection , the major recognized sequelae of campylobacteriosis are – Guillain –Barre Syndrome (GBS) is the most commonly reported chronic sequelae (Frickmann *et al.*, 2014)(Heimesaat *et al.*, 2014). Reactive arthritis (ReA) was also associated with *Campylobacter* post-infection (Rule, 2016)**.**

Irritable bowel syndrome , the Miller Fisher syndrome, a GBS variant, is also associated with preceding *Campylobacter* infection. Sequelae contribute significantly to the disease burden of campylobacteriosis (Frickmann *et al.*, 2014)

Post-infection irritable bowel syndrome (PI-IBS), characterized by chronic abdominal pain and bowel disturbances, has been reported to develop in ∼14% of patients suffering from *Campylobacter* enterocolitis with an odds ratio of 4 compared with uninfected controls from the same population. Symptoms of PI-IBS have been shown to persist for up to 8-10 years following an episode of enter colitis. Reactive arthritis, a type of spondyloarthopathy that primarily affects the knees, ankles, and feet, can develop in 3-5% of people after Campylobacter infection, as well as other gastrointestinal and genitourinary infections in human (Klem *et al.*, 2017)**.**



Figure(2-3): Environmental reservoirs, and clinical manifestations associated with *Campylobacter species*(Abdulazeez, 2022)

2.8.2 Clinical diseases and treatment options in animals

Campylobacter is found in a wide range of animal species. In most animals, it persists as an intestinal commensal without causing clinical disease, but in other cases, it can cause localized enteritis or systemic infections. Reproductive losses in ruminants (e.g., abortion and infertility) are among the most serious clinical disorders linked with Campylobacter infection in animals. The principal *Campylobacter* species involved with outbreaks of sheep abortions globally *are C. jejuni* and *C. fetal* subsp. fetus (CFF), and they also cause sporadic abortion in cattle and goats (Sahin *et al.*, 2017) .

Both organisms are frequently found in the intestine and gall bladder of healthy animals; however, in infected pregnant ewes translocation of *Campylobacter* across intestinal mucosa and systemic spread may occur, leading to fetoplacental infection and abortion, which typically happens in the third trimester of gestation. Historically, CFF was the primary *Campylobacter* species associated with ovine abortions worldwide, Vaccination is a frequent method for the prevention and control of *Campylobacter*-associated sheep abortion, but its effectiveness varies (BonDurant, 2005) .

Tetracycline is commonly used to control the disease, and tulathromycin has recently emerged as an alternate treatment due to concerns about tetracycline resistance in *Campylobacter* (Yaeger *et al*., 2020)

Infectious infertility, also known as bovine genital campylobacteriosis, is caused *by C. fetus subsp. venerealis* (CFV) and is an economically significant illness of cattle worldwide. (Klem *et al.,* 2017) .The bacterium lives in the genital tract of cattle and transmitted venerably to cows by carrier bulls.(El-Wadawe *et al.*, 2019)

Control and prevention involve the detection and removal of carrier bulls, as well as vaccination and antimicrobial treatment of bulls and cows (Michi *et al.,* 2016) . Control and prevention involve the detection and removal of carrier bulls, as well as vaccination and antimicrobial treatment of bulls and cows (Scallan Walter *et al.,* 2020).

2.9 Campylobacter detection and enumeration

2.9.1 Isolation of *Campylobacter spp.*

Thermo tolerant *Campylobacter* are micro aerobic in nature and are quite difficult to grow in natural environmental conditions, as they are sensitive to dehydration, freezing, and both low and high temperatures. For isolation of *Campylobacter* there are no gold standard methods, although various studies were conducted for the optimum growth of *Campylobacter spp.* from human, food, and other environmental samples. Various methods for isolation have been standardized by ISO and mention in ISO-10272, modification with time in procedure according to new research i.e. ISO- 10272-2006 and ISO-10272-2010. *Campylobacter spp.* are very fragile bacteria and require very care in handling during collection and transportation of samples from the field to laboratory. In order to avoid loss of *Campylobacter spp.* and protect the cell from drying and toxic effect due to oxygen, transport medium is used during transportation along with low temperature, i.e., at 4˚C (Jacobs-Reitsma *et al*., 2008)(Bertasi *et al*., 2016)

Obtaining a 1g sample, serially diluting it in maximum recovery diluent (MRD), and plating it onto modified cefoperazone deoxycholate agar (mCCDA) are typical procedures.(Bertasi *et al*., 2016)

However there are a wide variety of other media available to selectively isolate *Campylobacter* which contain various antimicrobial compounds to inhibit competing micro flora. These include Karmali, Preston, Butzler, Skirrows and Campy Brilliance agar and a summary of the antimicrobial

constituents in the media are presented as previously published by (Mughini-Gras *et al*., 2016)

Some of these media use charcoal or blood to bind oxygen, preventing it from having a toxic effect on *Campylobacter*, although some *C.jejuni* strains have been demonstrated to grow in-vitro with partial oxygen tensions of 21% (Mouftah *et al.*, 2021).Depending on sample type, an enrichment step may be necessary where low numbers or damaged cells may be present. (Corry *et al*., 1995).

However, whilst the ISO method recommends Bolton broth (Finkbeiner *et al*., 2006)it may not be optimal for all sample matrices. A review of the existing ISO method for food/animal feedstuffs (Finkbeiner *et al.*, 2006) by the Netherlands Food and Consumer Product Safety Authority (VWA) found that Bolton broth was not optimally selective for several sample matrices including chicken products. The review also found that the recommended ISO incubation conditions (37-42°C, 24-48hrs) were not optimal for all sample matrices (Mughini-Gras *et al*., 2016)

In some cases, a second enrichment step using Preston Broth has been recommended as an additional step to the existing ISO method (Papić *et al.*, 2017)**;** (Mughini-Gras *et al*., 2016). The report also concluded that plating onto mCCDA agar was optimal for the selective isolation of Campylobacter from all sample matrices (Mughini Gras *et al.*, 2012)

2.9.2 : Diagnosis of *Campylobacter spp*.

Campylobacter produces different characteristics colonies on different medium, which depends medium composition. Colonies of *Campylobacter* are usually round in shape produce gray to slightly pink colonies with or without metallic sheen on blood containing media and flat, glossy and grey to whitish colonies with or without metallic sheen on charcoal medium. They are gram-negative, motile, oxidase and catalyse positive, and negative for methyl red, acetoin and indole formation (Dingle *et al*., 2002) *Campylobacter spp.* has the ability to hydrolyse hippurate and rest of the species can't hydrolyse and this is helpful in differentiation of *C. jejuni* from other species (Mitchell *et al*., 2013 & Papić *et al*., 2017)

In taxonomic research, hippurate hydrolysis, commonly conducted by the quick tube test with ninhydrin as an indicator, was found to correlate closely with species distinction (Ghaffoori, 2017).

2.9.3. Confirmation

The *Campylobacter species* can be confirmed by both phenotypic and genotypic methods.

2.9.3.1. Phenotypic

Individuals' phenotype is a collection of observable characteristics that develop due as a result of their genotype's interaction with the environment. Thus, phenotypes represent the nature of an organism. Bio typing, serotyping, and phage typing are examples of phenotyping techniques (Fitzgerald *et al*., 2001)

Campylobacter spp. can be confirmed and classified by a variety of methods. Tests including conventional biochemical identification and confirmation, latex agglutination test, API Campy and serotyping. Biochemically *Campylobacter species* are gram-negative, small, spiral or comma shaped, motile organisms, they give positive results for oxidase, catalyse test and urease test. All species cannot hydrolyse hippurate except *C. jejuni* and all species are oxidase positive except *C. gracilis* (Pope *et al.*, 2007_Wulsten *et al.*, 2020 &Metreveli *et al.*, 2022)

Sometimes *Campylobacter species* are found in coccoid forms due to exposure to oxygen or using old culture for identification. Biochemical tests take long time and are replaced by other confirmation test due to its sensitivity, specificity and systemic errors. API *Campy* (Biomerieux France) is one of the confirmatory tests for *Campylobacter species*. This test consist 11 enzymatic, 11 conventional and 9 assimilation and inhibition tests. The sensitivity and specific for API Campy is found more than conventional in various studies and correctly identified *Campylobacter jejuni* (94%), *Campylobacter coli* (74%), *Campylobacter upsaliensis* (100%) and misidentified (5%) as other species (Bolton, 2015)while no significant difference found in conventional and API Campy methods (Pope *et al.*, 2007)

Lack of or difficulty achieving a calibration, lack of mobility, interaction between strains, and inability to distinguish between types ,are all drawbacks of these techniques. Furthermore, phenotypic techniques have a low discriminatory power (Stephan . L. W. On, 2013)

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2.9.3.2. Genotyping

In order to avoid errors in identification of *Campylobacter species* by both biochemical and serotyping methods, amplification of specific gene and specific region within species is carried out with the help of conventional as well as quantitate polymerase chain reaction (PCR). Highly conserved genes in specific species are used for amplification to correctly identify the species of *Campylobacter*. Some of the specific gene for amplification in various studies are: 16SrRNA for Campylobacter genus (Altekruse *et al*., 1999)

mapA and hipO for *Campylobacter jejuni* , cueE for *Campylobacter coli* (Denis et al. 1999), porA for *Campylobacter lari* and *Campylobacter upsaliensis*. Various genes were also used for genotyping i.e., cdtA, cdtB and cdtC (Wolffs *et al.*, 2007) cadF (Wolffs *et al.*, 2007) sapB and glyA (Gehua *et al.*, 2002), ipxA (Marsh *et al.*, 2010).

2.10 Molecular detection

2.10.1. Conventional PCR (polymers chain reaction)

The technology known as polymerase chain reaction (PCR) makes it possible to quickly and accurately detect species-specific DNA sequences. A DNA fragment is multiplied by multiple orders of magnitude during the PCR reaction. The method is based on a process known as thermal cycling, which repeatedly heats and cools a reaction to cause DNA melting and enzymatic DNA replication (Dieffenbach & Dveksler, 2003) . Conventional PCR, which is first developed in 1992 for the identification of chromosomal gene

sequences in *C. jejuni* and *C. coli*, may recognize cells in insufficient numbers and detect chromosomal gene sequences (Moore *et al.*, 2005)**.**This technique finds DNA in both living and dead bacteria after they have been grown and visualized(Reischl & Kochanowski, 1995_Humphrey *et al*., 2007).

2.10.2. Real Time PCR

Conventional techniques for isolation and identification of pathogenic organisms are quiet painstaking and time consuming process. Campylobacter isolation and phenotypic identification requires 5-7 days starting by enrichment followed by sub culturing, purification, and confirmation by biochemical and agglutination tests (Altekruse *et al.*, 1999) PCR is a rapid method for amplification nucleic acid for detection of organism (Snow *et al*., 1996), but it is also a complex process and required more labor and only gives qualitative results (Waage *et al.*, 1999).

Real time PCR is used nowadays for the detection and quantification of organisms in food products and for other diagnostic purposes. In real time PCR both internal and external control of known concentration are amplified in parallel with the sample of interest. The result of the unknown samples is calculated by comparing with results of control (Reischl & Kochanowski, 1995). Real time PCR assay is very sensitive and can detect very low number of organisms in sample e.g., 10-100 cells of *Campylobacter* present in a sample (Waage *et al.*, 1999) Different types of primers and probes are used real time PCR for identification of specific organisms. The results are calculated and measured in "real time" by monitoring change in florescence signals by amplified products. These are quantified by exponential phase of

amplification as the copies of DNA sequences become double after every cycle. The four phases can be distinguished in real time PCR; the lag phase, the exponential phase, the linear phase and plateau phase. All these phases can be monitored by computer screen. There may be bias occurred in PCR template to product ratios (Suzuki & Giovannoni, 1996).

2.10.3 PFGE

Pulsed field gel electrophoresis (PFGE), is a process of separation large DNA molecules in agarose gel with the help of electric filed which changes direction periodically. Pulsed field gel electrophoresis was one of the first method for DNA typing and are used commonly for rapid detection and investigation of food borne outbreaks, implemented by Pulse Net in USA for typing of important food borne pathogens (Adiguzel *et al.,* 2018)

PFGE is most commonly techniques used in epidemiological studies and it can separates large DNA fragment up to 10MB and time for unraveling and reorientation depends on the size of the fragment. PFGE was designed for the first time for the typing of *Campylobacter species* and is considered as a gold standard for typing of other foodborne pathogen, which was based on restriction digestion of chromosome into small number of large fragments with enzymes SmaI or Kpnl (Ahmed *et al.*, 2015) .

The results are analyzed by commercially software packages and the differences in the bands pattern is used between isolates for genetic comparison. Sensitivity of PFGE is depends on the enzymes used for restriction digestion and Kpnl is more appropriate than Smal for *Campylobacter jejuni* strains (Mukherjee *et al.*, 2013) Only one enzymes

SmaI is sufficient for showing differences among isolates but Kpnl must also use for similarity and confidence and discrimination between isolates (Altekruse & Tollefson, 2003).

This technique is time consuming taking 4-5 days and quite expensive, but still it is using for molecular typing of clone and closely related strains of *Campylobacter spp.* and tracking of other foodborne pathogens in poultry continuum (Sandberg *et al.,* 2015)

2.10.4. MLST

Multi locus sequence typing is one of the most commonly used methods for molecular typing of *Campylobacter species*. It is based on MEE (multilocus enzymes electrophoresis) and multiple genes are compared for nucleotide base changes in gene by different enzymes depending on mutation in their locus of gene (Maiden *et al.*, 1998) It is a highly reliable technique and internet based MLST databases which help in facilitating standardized nomenclature and rapid inter-laboratory typing scheme for result exchange. MLST is costly technique and still using for monitoring of *Campylobacter* species, it is based on variation in housekeeping genes ranging from 7-11 genes (Smith, 2016)for *Campylobacter jejuni* and expand for *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis* (Rule, 2016)It is highly recommended for *Campylobacter* population for understanding genetic variation than for investigation of outbreak (Kovanen *et al*., 2016)

Sequence data may be easily compared between laboratories and are well suited for electronic transfer and storage. Additionally, as nucleotide sequence determination from PCR results can be accomplished using killed-

cell suspensions, purified DNA, or clinical material, MLST can lessen the requirement to transport live bacteria. For the purpose of storing and exchanging data as well as MLST protocol information, a website has been set up at http://mlst.zoo.ox.ac.uk. The data can be used in the investigation of individual outbreaks, even though MLST is best suited to long-term and global epidemiology because it identifies variation that is slowly accumulating within a population. This is especially true when MLST data are combined with other data, like the nucleotide sequences of genes encoding antigens (Bygraves *et al.*, 1999 **;**Feavers *et al.*, 1999)**.**

2.10.5. RAPD

Random amplified polymorphic DNA (RAPD) is a typing method used for comparing relatedness the bacterial strains with generic PCR by amplifying random segment of DNA and band separation by agarose gel electrophoresis (Fan *et al.*, 1999 ; Sazali, 2020). RAPD has a high discriminating power and used for comparing the relation of Campylobacter species, but variation is different to control in this assay. RAPD has high reproducibility in one laboratory but inter-laboratory it varies (Misiewicz *et al.*, n.d.)

2.10.6. AFLP

Amplified fragment length polymorphism (AFLP) is used in epidemiological investigations of foodborne bacteria pathogen like *Campylobacter species* (Islam & Shinjo, 2009_Sazali, 2020). In this technique restriction fragments from genomic DNA is selectively amplified after restriction digestion by restriction enzymes. Multiple restriction present in bacterial DNA leads to high discrimination power of the isolates and the

mutation occurred in these genome is compared. Fluorescent lapelled or radioactive lapelled primer helps in automation and higher throughput. The product of AFLP is analyzed by polyacrylamide gel (Berge & Baars, 2020).

Restriction fragment length polymorphism (RFLP) identifies differences in homologous sequences of DNA after restriction digestion by restriction endonucleases, resulting in different length of fragments. In *Campylobacter*, RFLP is mostly conducted for flagella typing for detecting fingerprints of flagellin gene. Major falgellin gene (flaA) and minor flagellin gene (flaB) are the two genes that encode flagella in *Campylobacter*. The flaA gene are amplified, digested by endonucleases, and analyzed for characteristic pattern after the separation of fragments by agarose gel electrophoresis. This is economic, fast and easy technique but alone RFLP is not adequate for epidemiological typing due to its intra and inter genomic recombination with in flagellin gene. This technique, along with PFGE, is very useful for the differentiation of *Campylobacter* isolates from different origins (Mouftah *et al*., 2021)

2.11.7. Microarray

Microarray may be used for analysis of specific gene expression and evaluation of whole genomes. It may be used for differentiation and detection of *Campylobacter* species in bacterial population, investigating diversity and evolutionary pattern of stains (Altekruse *et al.*, 1999 ; Young, 2007; Twenge *et al.*, 2010). DNA microarrays bind to specific genes by complementary base pair hybridization to solid substrate. Sufficient amount of DNA form the test sample binds to sequences bounded into substrates (thousands replicates for

particular gene sequence) and makes whole genome sequencing by binding of so many replicated to one bound spot. The length open reading frames (ORFs) may be from 20 to 70 base pairs or 1000 base pairs in gene specific DNA fragments (Smith, 2016) Microarrays do not require DNA amplification. Although, it detects SNPs but are unable to detect novel genes. The results are quantified with the help of fluorescent dye (Cyanine) and scanned the intensity of wavelength of each spot in pixel units. It is an expensive, labor extensive technique and standardization problems between laboratories (Gascou *et al.*, 2014).

2.10.8. Whole Genome Sequence

Whole genome sequencing (WGS) provides high discriminating power to epidemiological studies by resolving pathogens that differ by only single base pair. WGS has ability to characterize pathogens but have for limited organisms and *Campylobacter jejuni* is one of them. *C. jejuni* was one of the first bacterial strains for whole genome sequencing and analyzed by next generation sequencing (NGS) technologies. Advances in NGS have made it possible to analyze WGS in outbreaks (Achtman *et al.*, 1999; (Tenorio & Flores, 2021) *Campylobacter spp*. have small genome of 1.6-1.7 Mb and can be easily sequenced (Narvaez-Bravo *et al.*, 2017). Beside these expertise and tools for bioinformatics are required for WGS.

This method was used to evaluate some schemes that have been implemented, and the discriminatory power is similar to that of classical serotyping, which is never sufficient for outbreak analysis. The discriminatory power can be adjusted by choosing the right number and type of genes as

markers. Long-term epidemiological research, however, might use it to describe genetic relatedness accurately (Mahdi *et al.*, 2022)

2.11 Antibiotic sensitivity

Three key uses for antimicrobial drugs are treating diseased people and animals, preventing disease in people and animals, and sub-therapeutically promoting growth in food animals. (Vuthy *et al.*, 2017)**.**

The broad and haphazard use of antibiotics in veterinary medicine, including food animal production, is one of the main contributors to the growth and spread of antimicrobial resistance (Marshall & Levy, 2011) (Mahdi *et al*., 2022)

Infections caused by drug-resistant strains required prolonged treatment, have a higher morbidity and fatality rate, and require higher treatment expenses. A remarkable increase in resistance among *Campylobacter* bacteria has been observed in recent years. This issue affects strains isolated from humans, animals, and food. One of the primary causes of this condition is the overuse of antibiotics. We chose azithromycin, erythromycin, gentamicin, ciprofloxacin, and tetracycline to estimate antibiotic resistance profiles in *Campylobacter* isolates since they are clinically utilized and often evaluated in both food/animal and human isolates. The medications of choice are macrolides (e.g., erythromycin) and fluoroquinolones (e.g., ciprofloxacin). Alternative antibiotics for the treatment of systemic *Campylobacter* infections include gentamicin, tetracycline, and azithromycin (Resistance, 2019).

In extreme cases, antibiotics may be used to treat *Campylobacter spp*. infections. The most widely used medications to treat campylobacteriosis in humans are erythromycin, fluoroquinolones, or tetracycline (Gahamanyi *et al.*, 2020).

The species of *Campylobacter* are on the World Health Organization's (WHO) list of global priority pathogens for antibiotic research and development. Several medicines are no longer effective in the medical treatment of campylobacteriosis, requiring the development of new medications and treatment strategies, because phytochemicals are a key source of bioactive substances with potent antibacterial properties, medicinal plants are promise in isolating candidate molecules for novel medicines in this context (Khameneh *et al.,* 2019) .

The disk diffusion method, in which the active component is dispersed into an agar plate of bacteria by a disk or by wells, can be used to assess the antimicrobial activity of a plant extract or of an antibiotic. The least inhibitory concentration (MIC), however, is the most effective technique to describe the antibacterial activity of a substance or extract. By using the broth micro dilution method, the lowest concentration of a substance is required to block the development of a microbe. For this reason, the plant extracts and compounds in this review with the strongest antibacterial activity were classified according to their MIC values. (Corry *et al.*, 1995); (Khameneh *et al*., 2019) ;(Hlashwayo, Barbosa, *et al.*, 2020)

2.12. Treatment of campylobacter

Most campylobacteriosis infections are self-limited and just need supportive care. For some enteritis instances, particularly severe ones, the antibiotics may be helpful (Meyer *et al.,* 2013)**.** In severe cases, antibiotics medicines called fluoroquinolones and macrolides (often erythromycin) are used to treat campylobacteriosis in severe cases (Gascou *et al*., 2014)**.**

Sensitivity testing is carried out to guarantee proper and prompt therapy (Rule, 2016).Antibiotics are occasionally administered, especially when the symptoms are severe or persistent; nevertheless, their use for enteric infections, especially those that are mild, is still debatable. Antibiotic medication is thought to be appropriate in HIV/AIDS patients with immunosuppressed individuals. Treatment with antibiotics can lessen the shedding of infectious germs (Meyer *et al*., 2013)**.**

However , Patients continue to excrete *Campylobacter* in their feces for several weeks to months after recovery, although the infection has been treated with antibiotics (Abd & Al-Nasrawi, 2015)**.** Due to the risk to human health, the National Antimicrobial Resistance Monitoring System (NARMS) in the US regularly gathers information on *C. jejuni* and *C. coli* isolates found in food animals at federally inspected slaughter and processing facilities, retail meats, and human clinical cases. (Olivier *et al.*, 2021)

Lab examinations Fluoroquinolone antibiotics, however, have been demonstrated to be ineffective against an increasing number of strains around the world, perhaps as a result of the use of chemicals associated with these drugs in chicken rearing (Roberts *et al*., 2008)**.**

A successful *Campylobacter* control method can involve vaccination, several populations have been shown to be immune to *Campylobacters,* including those who have already been ill, breast-fed children whose mothers have recently been exposed, and others (Zilbauer *et al.*, 2008).

2.13. Control Measures

2.13.1 Control & education

Control is mostly directed against the source of infection.Because consumption and handling of poultry consider the major source of infection ,the optimal way to control the number of human infection would be to limit contamination of poultry flocks(Abdulazeez, 2022), and by using of disinfectant footbaths ,food and water disinfection ,treatment the animal manure and isolation of ill contagiously (Zilbauer *et al*., 2008) . Efforts should be concentrated on practice designed to control and reduce levels of fecal contamination during live bird transportation ,slaughter and carcass dressing (Bolton, 2015).

Freezing at - 20˚C, treatment of wash water and irradiation are actual processing control to reduce contamination but irradiation changed the color and texture of chicken(Altekruse & Tollefson, 2003)Poultry products should be stored and transported at a temperature of 4˚C or lower to prevent proliferation of *Campylobacter* and other food-borne bacterial pathogens(Shane, 2000)

Although person-to-person transmission of *Campylobacter jejuni* infection is unusual, persons with any acute diarrheal illness should avoid preparation and handling of food until their illness resolves(Revez *et al*., 2014) **,** hands and utensils following contact with raw meat and poultry, and

proper cooking of chicken have been recommended as a prevention (Altekruse & Tollefson, 2003)**.** There is no certified vaccine against *Campylobacter* species (Olaimat *et al.*, 2014).

International travelers , immunocompromised individuals and pregnant women should pursue general safety measures to protect against diarrhea which include avoiding drinking of untreated water, unpasteurized milk and ingestion of undercooked meats and hand washing particularly in children whom in contact with diarrheic pets**;**(Altekruse & Tollefson, 2003)**;** (Olaimat *et al.*, 2014)and (Abdulazeez, 2022)**.**

2.13.2 Vaccine

There is no vaccine available against Campylobacter species. Scientists have made many attempts and are still working to overcome the problem in both animal and human origin. The burden of the pathogen can be decreased significantly by eliminating the organism with help of vaccination in poultry production system. Although vaccination for human population is not so important, it would help in reduction in mortality and morbidity in immune-compromised persons and travelers, respectively.

Chapter Three

Materials and Methods

3. The Methodology

3.1 Supplies and equipment.

The following table [3.1] shows the Supplies and equipment.

No.	Item of equipment	Company /origin
1	Autoclave	Hiraym _Japan
2	Anaerobic Jar	Oxiod / UK
3	Bench centrifuge	Hettich / Germany
4	Bezel burner	Iraqi
5	Biological Safety Cabinet	Labcono / USA
6	Cotton	China
7	Deep freezer – 20	Kw / Italy
8	Digital balance	Germany
9	DensiCHEK	CAPPB rare / UK
10	Dry microtubes incubator	Ae/ UK
11	DNA- RNA Spectrophotometer	APEL / UK
12	Electrophoreses	Cleaver / UK
13	Eppendorf tubes	CYAN-Cypress diagnostic / Belgium
14	Gloves	China
15	High-speed refrigerated centrifuge	Bio base / China
16	Incubator	Memmert / Germany
17	Light microscope	Germany
18	Microfuge IB Centrifuge	Hettich / Germany

19	Micropipette sets from 1µl to 1000µl	CAPPB rare / UK
20	Micropipette tips (different sizes)	China
21	Kern PFB balance	Kern & Sohn / Germany
22	Optimus 96G thermal Cycler	QLS / UK
23	petri dish	China
24	plane tubes	China
25	Plastic rack	China
26	Refrigerator	Kw / Italy
27	Standard loop 0.01	China
28	Sterile syringes	China
29	Swabs	China
30	UVP	Analytik Jena / UK
31	Water bath	Memmert / Germany
32	Water distillate	Memmert / Germany
33	Vortex mixer	Memmert /Germany

3.1.2. The Chemicals

Table (3-2) shows chemical materials using in the work.

	The Chemical Materials. Item	**Company/ Origin**
1	Gram stain	AFCO / Jordan
2	Catalase	Hardy / USA
3	Oxidase	Hardy / USA
4	Indoxyl acetate	Hardy / USA
5	Primers	IDT / Canada
6	Safe-Green 100bp Opti-DNA Marker	ABM / Canada
7	Agarose LE	Intron / Korea

8	RedSafe nucleic acid staining solution	Intron / Korea
9	TEB buffer 10 X	Promega / USA
10	Loading dye	Kapa / USA
11	Glycerol	Oxiod
12	Campylobacter selective supplement	Karmali / UK
13	Antibiotic	Oxiod
a	Azithromycin	15 mg / oxiod
b	Ciprofloxacin	5 mg/ oxiod
c	Ceftriaxone	30 mg/ oxiod
d	Nalicdixic acid	30 mg/ oxiod
e	Gentamicin	10 mg/ oxiod
f	Penicillin	10 mg/ oxiod
h	Cephalexin	10 mg/ oxiod
i	Tetracycline	30 mg/ oxiod
j	Imipenem	10 mg/ oxiod
k	Ampiciline	10 mg/ oxiod
l	Amoxicillin	25 mg/ oxiod
m	Streptomycin	10 mg/ oxiod

3.1.3. The kits

Table (3-3): PCR kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	G-Spin Bacterial Genomic DNA Extraction Kit	Intron	Korea
	Proteinase K		
	Lysis buffer (SL)		
	Binding buffer (ST)		
	Washing buffer 1 (W1)		
	Washing buffer 2 (W2)		
	Elution buffer (E)		
	GD column		
	Collection tube 2ml		
	Nuclease free water		

3.1.4. Culture Media

The table (3-4) shows the culture media used in this study.

	Item	Company/ Origin
1	*Campylobacte*r agar base	Hardy / USA
2	C&S medium Cary Blair	MCC / USA
3	Brain heart infusion broth	Oxoid / UK
5	Modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA)	Oxoid / UK
7	Makoncy agar	Oxoid / UK
8	Mullar Hinton Agar	Oxoid / UK

3.2. Methods of Samples Collection and Diagnostic.

3.2.1. Study design and sample collection:

3.2.1.1. Study design

A study was done to determine the prevalence of Campylobacter species in humans ,milk and milk product at Karbala retail points, 100 samples of milk collected from different farms ,fields and local store distributed in Karbala, also100 samples from milk product as cheese 40 sample ,cream 20 sample and 40 sample of yogurt, finally about 100 stool samples from people with diarrhea from Children's and Al-Hussein educational hospital at Karbala province in Iraq .The samples as shown in figure (3-1) were collected during a period from October 2022 to march 2023.

Table (3-5): Sample Details

Milk		**Milk product**						**Human**	
100		**100**						**100**	
Raw milk		**Cheese**		**yogurt**		**cream**		**Age Below 10 year**	
Farm and field	**Local store**	**Street vender**	**Local store**	**Street vender**	**Local store**	**Street vender**	**Local store**	**Under 5 year**	**above 5 year**
50 Sample	**50 sample**	**20 sample**	**20 sample**	**20 sample**	**20 sample**	**10 sample**	**10 sample**	**50 stool sample**	**50 stool sample**

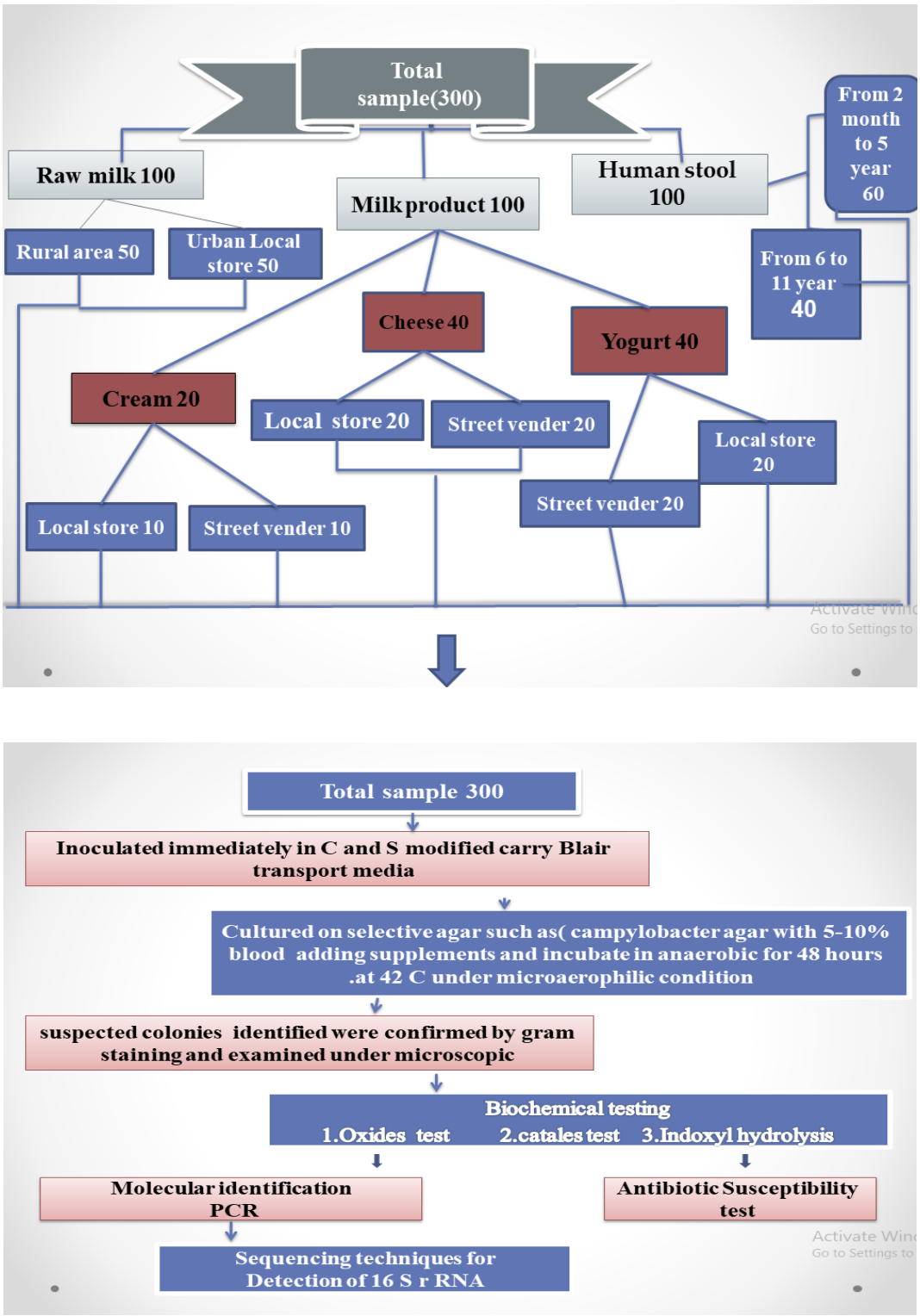


Figure (3-1): The study design for diagnosis and analysis of *Campylobacter spp.*

3.2.1.2. Collection of samples:

A. Collection of Human samples:

Diarrhea is the main sign for clinical diagnosis. The diarrheal illness with *Campylobacter spp.* may be watery, mucoid or quite severe bloody (Ali, 2008). Diarrhea defined as the passage of three or more than three loose or watery stool in 24 h, or passage of one or more bloody stool, watery diarrhea, defined as semi-formed to loose or watery, without the presence of blood, while mixture of stool with blood macroscopically referred as bloody diarrhea (Manatsathit *et al*., 2002)

A total of (100) stool samples from patients suffering diarrhea with ages ranging from 2 month to 10 years were collected in sterile containers from Children's Hospital and Al-Hussein educational hospital at Karbala province in Iraq during a period of six months from October 2022 to the end of march 2023 after clinician consultation and microscopically examination in the hospitals where many samples contain motile bacteria, pus and few contain mucous and blood .

Diarrhea was defined as watery (85), mucous (6) and bloody (9) depending on the appearance of the stools . About one gram of stool was Inoculated immediately onto C&S modified Carry Blair transport media then Cultured on Campylobacter agar enriched with 5-10% blood and Incubated in anaerobic jar for 48 hrs. at 42°C under microaerophilic condition.

B . Sample Collection of raw milk and milk product.

Collecting milk, cheese ,cream and yogurt samples from street vendors and shops in various areas of the Holy Karbala Governorate. The sample was

taken during sampling and preserved in a sterile screw-capped vial that was kept in an ice-filled ice box .Packets and transported right away to a bacteriological microbiology lab analysis. Isolation and bacterial identification were carried out using the methods

3.2.2. Preparation of Media

3.2.2.1 *Campylobacter* Selective Agar

Campylobacter agar base (Criteron, Hardy Diagnostic) medium was used for selective isolation of *Campylobacter* from samples. The medium was prepared by suspending 37 g after adding 1000 ml of distilled water, autoclaved according to manufacturer's guidelines, cooled to 45 °C, then enriched with 5-10 percent (v/v) human blood, and simultaneously, *Campylobacter* selective supplement (Karmali) was added aseptically, mixed well and dispensed into sterile plastic petri dish.

3.2.2.2. Brain Heart Infusion Broth.

Dissolved 37 g of BHI in 1 litter distilled water. Sterilized by autoclaving at 121°C for 15 minutes.

3.2.2.3. Muller – Hinton Agar

The medium was prepared according to the manufacturer’s instructions, and after sterilization and cooling to 45 °C, 7% of blood was added to it, then poured into sterile dishes. This medium was used in antibiotic susceptibility testing (Abd & Al-Nasrawi, 2015)

3.2.3. Gram Stain Reagent Kit.

Gram stain reagents and this involve a number of reagents: crystal violate stain, lugol's iodine, alcohol acetone and safranin stain is used to determine the type of bacteria in a sample whether it positive or negative. In order to examine *Campylobacter* colonies microscopically, the colonies were picked up, a glass slide was stained with gram stain, and a lens was immersed in oil to see them. (Goldman & Green, 2015)

3.2.4. Biochemical Identification tests.

3.2.4.1. Catalase test.

Placing a few drops of H_2O_2 (3%), on a sufficient amount of well isolated culture, mixed to facilitate the reaction, immediate appearance of bubbles was an indicator of positive result(Reiner, 2013).

3.2.4.2. Oxidase test.

Moisten a part of the oxidase test strip with a drop of water in petri dish. Then loop full of pure suspected colonies was spread over the strip with the aid of wooden stick, colonies should be from 48 hours old, the color change to purple within 30 seconds to be recorded as positive (Shields & Cathcart, 2010).

3.2.4.3. Indoxyl test.

Manufacturer's instructions were followed to accomplish the Indoxyl acetate disk hydrolysis. The disks were equilibrated to room temperature prior

to use. one drop of sterile water was added to the disk and placed in a petri dish or glass slide. The disk should not be saturated, then, it inoculated with a heavy inoculum (a few colonies) harvested from a pure culture of the testing organism grown for 24-48 hours. For at least 30 minutes, incubate discs aerobically at room temperature, and evaluate for color development.

If the color changed blue or blue-green within 20 minutes it was interpreted as positive result. While weak positive result was considered when pale color developed within 10-30 minutes. However, negative recorded when no color change happened within 30 minutes (Cerna-Cortes *et al.*, 2012).

3.2.5.Macfarland solution

This solution was prepared according to (Ristaino *et al*., 2021)as follows:

1- Solution A: this is Prepare by dissolving 1.175 g of barium chloride powder (BaCl2) in 90 ml of sterile distilled water, then add the volume to 100 ml with distilled water.

2- Solution B: Prepared by mixing 1 ml of concentrated sulfuric acid (H2SO4) with 99 ml of sterile distilled water. Different concentrations were prepared from it to control the concentrations of bacterial plankton.

3.2.6. Molecular methods

3.2.6.1 DNA extraction

All phenotypically identified bacterial isolates were subjected for DNA extraction by using G-spinTM Genomic DNA extraction kit (Intron/Korea) **.**

3.2.6.2 Preparing the primers

According to instruction of the primer synthesizer company, the primers (originally lyophilized), were dissolved in the free ddH2O to obtain a final concentration of 100 µM/µl prepared from the stock primers to be used as a work primer(Linton, 1997)

Primers used in this study

Table (3-6): Primers for general detection of *Campylobacter*

Organism		Target gene	Sequence (5'-3')	Ta (°C)	Product size	Reference
Campylobacter spp.	**16S rRNA**	**F**	**GGATGACACTTTTCGGAGC**	**56**	**812 bp**	(Abdulazeez, 2022)
		R	**CATTGTAGCACGTGTGTC**			

3.2.6.3 PCR mixture preparation and amplification of 16S rRNA gene for molecular identification of genus-*Campylobacter.*

PCR was conducted in a 50 µl volume containing 4 µl of bacterial DNA, 4 µl of each Forward and Reverse primer pairs, 25 µl of GoTaq® G2 Green Master Mix (Promega/USA), and the volume was completed to 50 µl with molecular grade water, all these were mixed in Eppendorf tube and spun

for 30 seconds then placed in Optimus PCR-96G thermocycler (QLS, UK). The condition for the reaction was revealed in Table 3-9.

Table (3-7): PCR conditions

Phase	Temp. (°C)	Time	Cycles
Initial denaturation	94°C	5 min	1X
Denaturation	94°C	30 sec.	35X
Annealing	56°C	30 sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1X

3.2.7 Electrophoresis

Following the loading of Safe-Green 100bp Opti-DNA Marker and the samples, the electrophoresis system was set as following: 90 Volt, constant current, 45 minutes' time. Finally, the gel was transferred into UVP system to observe the PCR products under 320nm UV light source.

3.2.7.1 Agarose Gel Electrophoresis of DNA

he electrophoresis was performed either to detect the presence of extracted genomic DNA or to determine the size of the PCR product

3.2.7.2. Preparation Agarose Gel

The preparation of agarose gel was performed according to the protocol that was described by (Sambrook et al., 1989). Briefly, 1 gm of agarose was dissolved in about 100 ml of a 1X TBE buffer and heated to be boiled in a

microwave machine. Following a cooling step at 45-50 °C, 5 µl of Red Safe nucleic acid staining solution was added to the gel and poured into the gel cast and left for about 30 minutes to be completely solidified. The gel-plate was transferred into the gel tank and filled with 1X TBE buffer to point of covering the gel surface.

3.2.7.3 Loading the PCR products

About 5 µl of each PCR products was dispensed into the middle of loading well. About 5 µl of Safe-Green 100bp Opti-DNA Marker was added to the first hole in the lines of the gel to be served as a marker for measuring the size of the PCR products.(Mahdi et al., 2022)

3.2.8 Sequencing and Analysis of the 16 S r RNA

DNA sequencing of 16S rRNA gene (812 bp) products of 20 Campylobacter spp. isolates that randomly selected, were carried out by sending 50 µl of forward primer and 50 µl of amplicon of above gene to Bioneer Company (Korea). The results of DNA sequencing were analyzed using MEGA 7 with NCBI for detection of phylogenetic tree and alignment. The data were compared with DNA sequencing data available at gene bank in NCBI.

3.2.8.1 Accession Number

The received gene sequencing for each strain was submitted to NCBI gen Bank Database for registration the sequence and receiving accession number.

3.2.8.2 Phylogenetic Tree Reconstruction

The distance of evolutionary were calculated using the Neighbour-joining (Jerome *et al.*, 2011) MEGA5 software was used to analyze evolutionary data (Kumar *et al.*, 2016). Phylogenetic trees were inferred using neighbor-joining with bootstrap analyses based on 500 replicates. A similarity index was generated using phylogenetic tree constructs and compared with known sequences against the GenBank database.

3.2.9 .Maintaining the *Campylobacter* bacterial isolates (Long-term storage method)

In order to maintain pure isolates and keep them for a long time, *Campylobacter* was cultured on a brain heart infusion broth (BHI). Then, about 0.8 ml of the cultured broth is taken and mixed with 0.2 ml of glycerol. Finally, kept in a freezer at -20°C until use (Vandepitte, 2003)**.**

3.3. Susceptibility Test for Antimicrobials Using Disk Diffusion (DD) Method (CLSI, 2020)

Step 1: Preparation of Inoculum

Few colony isolates were subculture on Campylobacter agar with 5% sheep blood and incubated for 48 h at 42 °C in a micro-aerophilic atmosphere visually with McFarland standard 0.5%.

Step 2: Culturing of Petri-Dishes

A sterile cotton swab was inserted into the direct suspension and squeezed well on the inner wall of the tube in order to remove excess fluid.

Then Muller-Hinton agar plate was then inoculated using the streaking method across the whole agar surface more than three times.

Step 3: Application of the Antibiotic Discs

The discs were placed over equal distances between each disc on the agar plate with a size of 90mm. Then, incubated in an inverted position at 37°C.

Step 4: Reading the Results

The inhibition zone diameter was calculated after incubation for 18 hours.

3.4. Statistical Analysis

For this study, SPSS (version 21) was used as the statistical software. An analysis of Chi-square scores determined differences between the one group. A significant difference was determined to be five percentages ($P \leq 0.05$) (Cohen,1960)(Benjamini&Hochberg,1995)(Field,2013).

Chapter

Four

Result

And analysis

4. Results

4.1. Isolation and Identification of *Campylobacter spp*.

the current study is conducted to find out the prevalence of infection and the bacterial distribution in human, milk and milk products respectively, that is illustrated in table (4.1) indicates that total of 200 samples of local fresh milk and milk product (raw milk, yogurt, cream and cheese) collected from different location of Karbala province and as follow 100 sample of human stool sample from educational children hospital & Al-Hussein educational hospital, the sample infected with *campylobacter spp*. were isolated (18%, 9% and 11%) samples respectively

Table (4-1): show the percentage of isolation of campylobacter from human , milk and milk products.

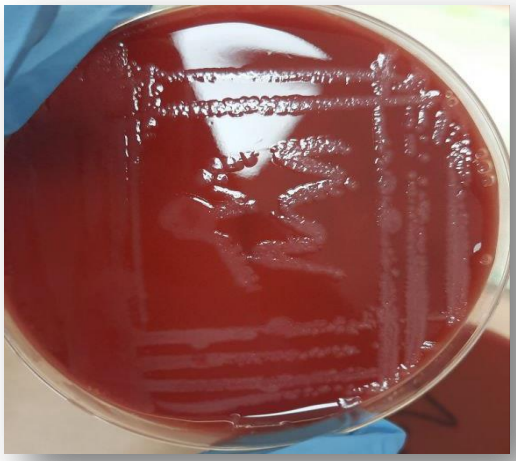
Type	**No. of sample**	**Positive**	**Percentage**
Milk	100	18	18%
Milk product	100	9	9%
Human	100	11	11%
Total	300	38	12.6%
Statistically analysis	Chi square= 3.565 P= 0.168		

The infection rate with *Campylobacter spp*. (12.6%) was non significantly ($p<0.05$). The results had similarity with these appeared by the study of (Almashhadany, 2021) which was 12.6% of raw milk samples contaminated by *Campylobacter species* in Erbil-Iraq retail markets.

Also the isolates had an agreement with (Flink & Nyberg, 2020) who found isolation ratio of campylobacter spp. , shiga- toxin producing *Escherichia.coli* (STEC)_thermotolerant and *Salmonella* spp. in Swedish dairy.

4.1.1 Cultural Characteristics:

the culture was done on the selective media for *Campylobacter spp*. and incubation in low oxygen conditions at a temperature of 42°C for 48-72 hours. Its edges were mostly wet and formed a merging line along the planting line, which gave the colonies a tailed appearance ,and this was consistent with what he described (Abdulazeez, 2022)as show in(figure4-1).



Figure(4-1): morphology of *Campylobacter* Colony on *Campylobacter* base agar with human blood.

The samples used in this investigation were cultured on enriched and selective media (*Campylobacter* agar base), which also contained 5% blood and a *Campylobacter* selective supplement made up of sodium pyruvate, cefoperazone, vancomycin, and cycloheximide The plates were incubated at 42°C for 48 h under micro aerobic conditions (about 80%-90% N2, 5%-10% CO2, 5%-10% O2) in a sealed jar using gas packs (Abdulazeez, 2022)

The culture of *Campylobacter spp.* isolates on selective media showed that all colonies are small, mucoid, usually grayish to creamy grey in color, slightly raised, moist and often produce discrete colonies, flat with irregular edges, and non-hemolytic as shown in Figure (4-1). The findings were similar to the results of (De Guevara *et al*., 1989)**;**(Hasso, 2018) (Asmat & Khan, 2020) and (Almashhadany, 2021)**.**

Campylobacter is fastidious organisms that require complex media containing blood for growth under in vitro conditions (Christidis *et al.*, 2016)**.** It is difficult to isolate this organism from stool samples without the use of selective techniques (Huang *et al.*, 2015). The selective media was used in order to inhibit the growth of more rapidly growing components of the enteric bacterial flora and to inhibit yeasts and molds because Campylobacter species multiply much more slowly than other enteric bacteria and in order to increase the success rates of *Campylobacter* isolation (Revez *et al*., 2014) (Oyarzabal & Carrillo, 2017)**.**

There are several antimicrobial combinations that were formulated to be used as selective agents in media used for the isolation of Campylobacter from samples (Chon *et al.*, 2020)**,** These antibiotics were selected because *Campylobacter spp.* are naturally resistant for them but they vary in the degree of inhibition of contaminating flora(Chon *et al.*, 2020)**.**

4.1.2 Microscopic Identification

Campylobacter Spp. colonies were examined under the microscope to reveal that the cells were Gram negative and had a variety of pleomorphic shapes, including the convex stick shape from which the organism got its name (curve = campylo means convex and rod = bacter means stick), the

shape of the letter (s), and the shape of a gull wing. wings in the manner depicted in figure (4-2). this result is agreement with (Perez-Perez & Blaser, 1996) and (Barakat *et al.*, 2015)(Abdulazeez, 2022)**.**

For several reasons, including bacterial strain, basal medium, moisture level on the agar surface, incubation temperature, and incubation time, colony morphology of *Campylobacters* was used as a guide for identification but has not been used as an important distinguishing factor (Aldarraji,2018).

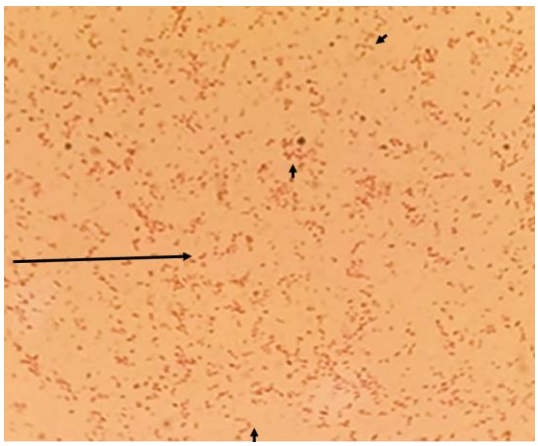


Figure (4-2): morphology of *Campylobacter spp.* under microscope with magnification power 100X.Arrow: curved form; Arrowhead: sea-gull winged form with magnification power 100X.

Campylobacter spp. contain a layer of lipopolysaccharides (LPS), which prevent absorption of crystal violet stain and accept safranin red color stain after removing the lipopolysaccharides layer with acholine. Microscope examination revealed that the *Campylobacter* bacteria appeared as gram negative bacilli after staining with Gram stain.(Helmy *et al.*, 2017)**;**(Isabel, 2019)**.**

Gram staining was used to visualize the probable bacteria under the microscope. The results revealed tiny, spirally curved, mobile that were gram-

negative. According to published research, *Campylobacter* cells are curved rods (Abdulazeez, 2022)**.**

4.1.3 Biochemical Test Results.

Thirty–eight isolates out of 300 samples were confirmed using gram staining to be *Campylobacter* species with positive oxidase, catalase and indoxyl acetate test .

Table (4-2):Identification of *Campylobacter* based on specific biochemical test

Number	Test	No .of suspected sample	Response
1	Gram stain	38	Gram negative
2	Catalase test	38	positive
3	Oxidase test	38	positive
4	Indoxyl test	38	positive

These bacteria produce catalase to reduce the toxic product hydrogen peroxide (H2O2) created in their environment, while the presence of cytochrome C, a compound that helps transfer electrons to the cytochrome oxidase complex make the genus members oxidase-positive As in figure(4-3)(Arafa *et al.*, 2017).

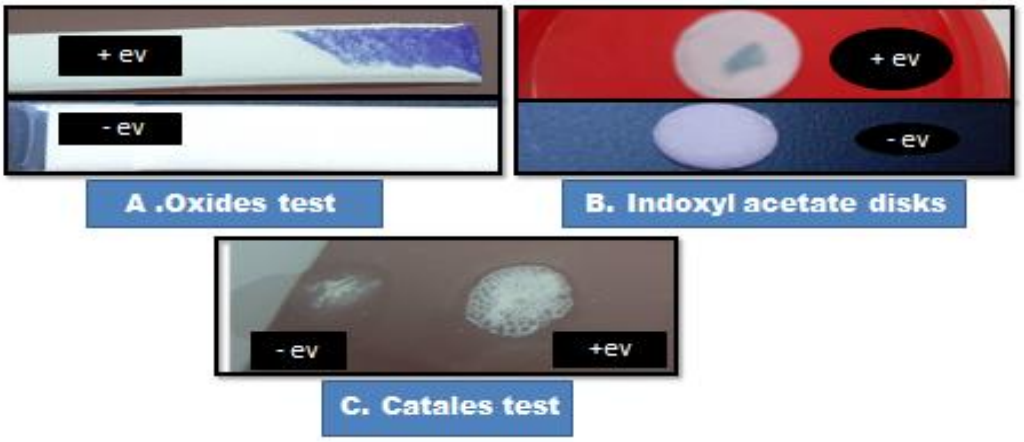


Figure (4-3): biochemical tests of *Campylobacter*. a) catalase test; b) oxidase test ; c) Indoxyl test

Biochemical tests (catalase, oxidase and indoxyl) were achieved on the suspected colonies and the results were positive for all the thirty-eighty suspected isolates.

These bacteria produce catalase to reduce the toxic product hydrogen peroxide (H_2O_2) created in their environment, while the presence of cytochrome C, a compound that helps transfer electrons to the cytochrome oxidase complex make the genus members oxidase-positive. Oxidase enzyme is existent in most members of the Campylobacteriaceae and all Helicobacter (Mahdi *et al*., 2022)

The present results are compatible with the phenotypic identification reported by others (Kanaan & Khashan, 2018)(Lanzl *et al.*, 2022)**.**

It was also found that *Campylobacter* was Oxidase-positive due to the presence of cytochrome C a function in the bacteria as a key participant in the life-supporting function of ATP synthesis (Marinou *et al.*, 2012) (Lanzl *et al.*, 2022)

Also, a large proportion of the bacteria was positive for Indoxyl acetate test, the presence of esterase enzyme present in *Campylobacter spp.* organisms can be confirmed in vitro by the bacterial hydrolysis of indoxyl acetate to release indxyl ,then combines with oxygen to spontaneously form indigo and Indoxyl Acetate Disk test becomes blue as showed in Figure (4-3) (Hodge *et al.,* 1990)**;** (Abdulazeez, 2022)**.**

4.2. Prevalence of *Campylobacter spp.* According to Sample Sources.

4.2.1. Prevalence of *Campylobacter spp.* in raw milk and milk product.

Campylobacter is an important gastrointestinal pathogen causing worldwide outbreaks according to The European Centers for Disease Control and Prevention (ECDC) and the Global Enteric Multicenter Study (GEMS). The current study recorded 27 (13.5%) positive results distributed between milk and milk products . These findings are concor with a prior study conducted in Pakistan, which discovered that butter and raw milk had the greatest concentrations of *Campylobacter* (Mahmood *et al.*, 2009). Additionally, similar prevalence rates ranging from 12% to 18% were also reported from Italy (Bianchini *et al*., 2014)**,**Tanzania(Kashoma *et al.*, 2016), and from Erbil_Iraq 12.6% (Almashhadany, 2021) **.** However, lower rates were also reported in other studies from Iran (6.25%) (Rahimi, Sepehri, *et al.*, 2013), Turkey (7.2%) (Elmalı & Can, 2019)Egypt (4.44%) (Barakat *et al.*, 2015), and India (2.9%) (Modi *et al*., 2015).

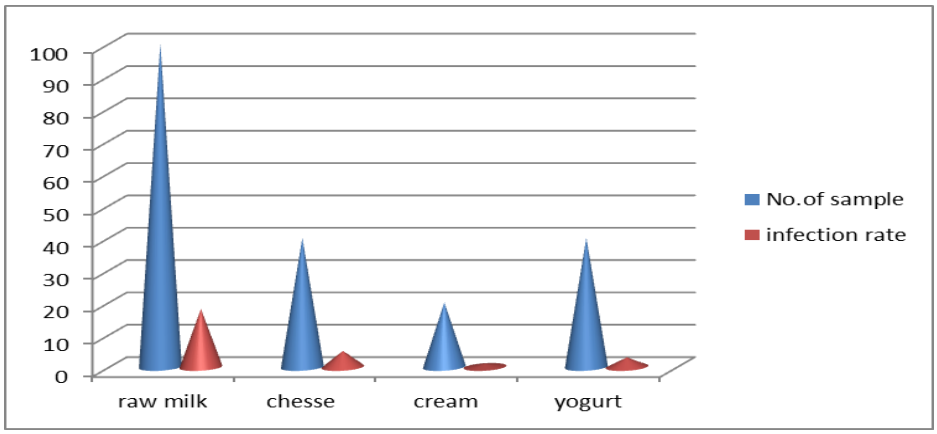
These variances could be explained by a variety of reasons, including regional differences, the sensitivity of the detection method, levels of hygiene, eating practices, and the presence of natural *Campylobacter* reservoirs (El-Naenaeey *et al.*, 2021).

Table (4-3) : distribution of *Campylobacter* in milk and milk products

Type	NO. sample	Positive	Percentage
Raw milk	100	18	18%*
Cheese	40	5	12.5%*
Cream	20	1	5%
Yogurt	40	3	7.5%
Total	200	27	13.5%
Statistical analysis	Chi square= 26.188 *P=0.0001*		

*** Significant differences**

the current results shown, it turns out that there is a correlation between the bacterial existence and the type of sample where the evidence showed significant increase in milk when compared to milk products (p-value ≤0.05) figure(4-4).



Figure(4-4) prevalence of *Campylobacter spp*. In raw milk and milk products .

High water activity, excellent nutrient content, and appropriate pH make milk a suitable media for the growth of several microorganisms like *Campylobacter* bacteria, despite being sterile when secreting from the udder ,the more frequent second source of campylobacteriosis is raw milk (Taghizadeh *et al*., 2022)

Popularly, consuming organic and raw food has been increased, so consumers need to be aware of the danger related to consumption of unpasteurized milk (El-Naenaeey *et al.*, 2021)The high occurrence of *Campylobacter spp*. in raw milk in the current study could be due to environmental contamination of milk during or after milking with infected animal wastes or from contaminated external surface of the teats, unsanitary equipment or hands of workers and storage practices(El-Naenaeey *et al.*, 2021)

Raw milk is a potential source of contamination for cheeses ,that is manufactured from raw cow's or buffalo's milk in farmers' houses. The presence of the most positive among the cheese samples is explained by the use of raw milk in the production process and the unsanitary handling, storage, and marketing practices. The prevalence of *C. jejuni* in this study is higher than that mentioned by (Barakat *et al.*, 2015)(El-Zamkan & Hameed, 2016) investigation, which was able to extract *C. jejuni* from 6.7% of the samples, while (El-Sharoud, 2009) failed to find any *Campylobacter spp*. of examined cheese samples.

4.2.1.1 Prevalence of *Campylobacter spp.* In raw milk & milks products according to the Residency Place.

Table(4-4):Infection Rate with *Campylobacter spp.* according to Residency Place

Residence place	Number of sample	Number of positive sample	Positive percentage
Rural area	100	19	19% *
Urban area	100	8	8%
Total	200	27	54%
Statistical analysis	Chi square =5.085 P=0.024		

*There are significant differences among rural and urban regions at ($p<0.05$).

As result from table ,the infection rate of *Campylobacter spp.* was higher in rural regions (19%) numerically than in urban regions (8%) according to the residency type as listed in figure (4-5)

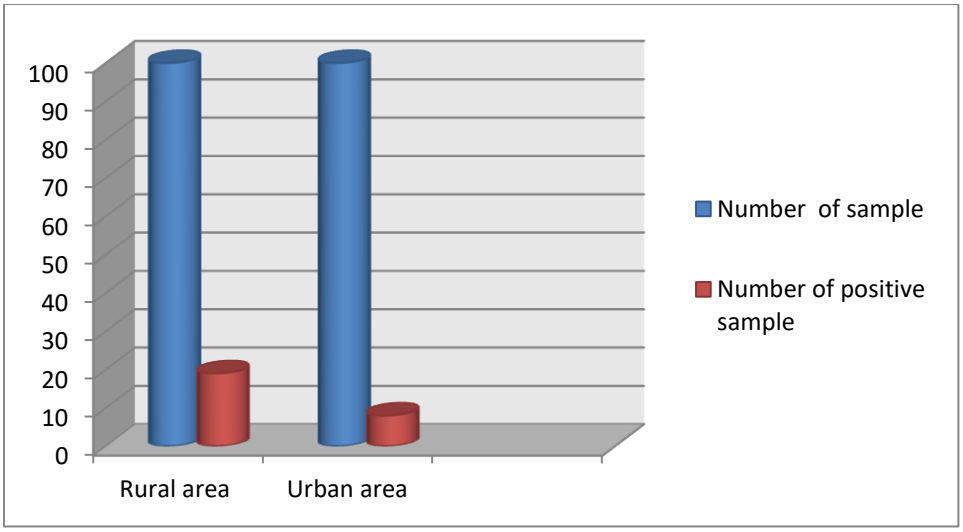


Figure (4-5): Prevalence of *Campylobacter spp.* According to the Residency Place

The detection rate according to residency place in present study agreed with (Al-Mawla *et al.*, 2008)who reported that rural area was found to be significant factor in developing *Campylobacter spp.* , infection is more prevalent in rural regions than urban regions (Levesque *et al*., 2013)**;** (Gölz *et al*., 2014)(Connor *et al.*, 2020)**;**(Indykiewicz *et al.*, 2021) and (Benshak *et al.*, 2023)**,** in contrast to the finding of (van Hees *et al.*, 2007) **;**(Tjang *et al.*, 2007)**;**(Jore *et al*., 2010) and (Kim *et al.*, 2023)who found that the incidence in urbanized area was more than rural areas.

The results of present study differ in infection rates according to residency where significant difference between rural and urban areas and may be the distribution of *Campylobacter spp.* infection depend on the degree of urbanization of the residency place, where exposure to risk factors such as ;contact with farm animals, animal stool, consumption of raw milk and untreated water may cause increasing incidence of *Campylobacter* infection .

4.2.2 prevalence of *Campylobacter spp*. in Human Stool Samples.

Samples collected from infected patients who suffered from diarrhea and some of them suffered other symptoms such as fever, colic and vomiting ,the sample was taken from educational children hospitals in Karbala city ,as in figure (4-6).

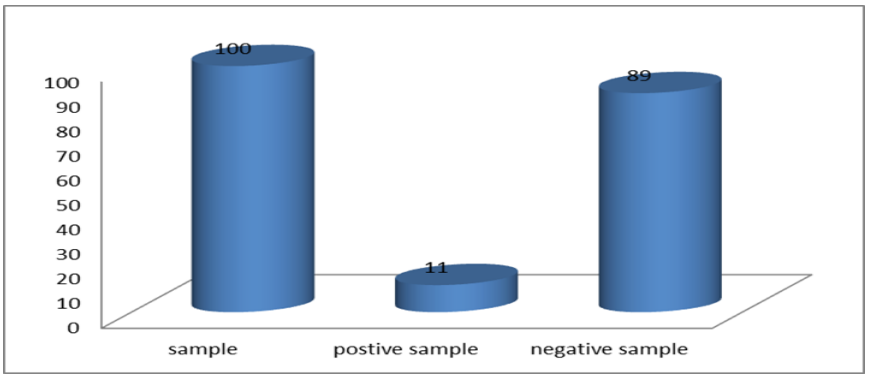


Figure (4-6): Percentage of *Campylobacter spp*. in the patients stool samples

This result was agree with (Reddy & Zishiri, 2017)**,**who identified *Campylobacter spp*. from stool samples by PCR assay, while much higher than that found by (Alemka *et al.*, 2013), who identified *Campylobacter spp.* in patients suffered diarrhea with (16.6%) by direct Real-Time PCR by hipo gene primers and proved that PCR assay was sensitive (100%) in comparison with (49%) sensitivity of direct bacterial culture . The different in detection rate in comparison with other studies may influenced with many factors such as age , season, geography and immune state of human (Teske *et al.*, 2013)

This overall recovery rate of *Campylobacter spp.* was compatible with the study in al Diwanyiah governorate by AL-Hamadani and Saleh 2011 who found that the percentage of *C.jejuni* was (9%) in children with positive *C.jejuni* culture. The prevalence of isolation *Campylobacter* species among diarrheic children was reported to be 5.4% in Turkey (Eryıldız et al., 2022) Seven percent in India (Mukherjee *et al.*, 2013) and 11.1% in Lebanon (Rafei *et al.*, 2019)**.** As reported by WHO and FAO 2012, the incidence of Campylobacteriosis was 9.3 in Europe America. *Campylobacter* is 1 of 4 key global causes of diarrheal diseases. It is considered to be the most common bacterial cause of human gastroenteritis in the world (Barati *et al.*, 2021)

Low recovery rate by culture method may be due to fastidious nature of bacterium, self-limitation of *Campylobacter* and may be involved several factors which are difficult to control, causes difficulty with the culturing of the organism, such as contamination , presence of intestinal flora, loss of viability of organism during transportation as well as intake of the proton pump inhibitors (PPI) which have indirect antibacterial effect. All these may be responsible for a negative predictive value associated with culture of campylobacter (Abdulazeez, 2022)

In Iraq ,several studies investigated Campylobacter enteritis in children and identified the pathogen with different percentages; in Baghdad thermophilic Campylobacter has been isolated with 10% from patients ranged from 2 months to 7 years (Saliih & Al-Saad, 1994)(Al-DOORI *et al.*, 2022).In Al-Basra, *Campylobacter jejuni* isolated from diarrheic patients ,their ages ranged (43day-11year) with (13.8%) (Mohammed *et al.*, 2004)**,**(Terefe *et al.*, 2020). In Al-Ramadi , *Campylobacter* species isolated with (8.92%) from children under 5 years (Al-Ani *et al.*, 2008)(Huda, 2016).

In Al-Diwanyiah ,thermophilic *Campylobacter* isolated and detected by conventional PCR assay with 33.3% from children under 2 years (Al-Hisnaway, 2008)The difference in the results of Iraqi provinces studies with this study may be attributed to sampling, season ,age and detection technique such as culturing ,conventional PCR in previous studies and Real-Time PCR in present study. Studies from other countries reported the isolation rate of *Campylobacter jejuni* in children with different percentages from Iran with (9.8%) in (1-12 year) children (Jazayeri Moghadas *et al.*, 2008)(Khademi &

Sahebkar, 2020), (18%) from children up to 12 year in Pakistan (Hussain et al., 2007)

The high incidence rate in children may be interpret due to incomplete immune response as well as to a variety of infectious diseases, close contact with animals or the environment and lacking sanitary awareness. It has been suggested that innate immune responses are not static but change with age, where there are risk periods for particular infections in early life and in older adults (Kollmann *et al.*, 2012).

An Australian study reported that infant and young children are at particular risk of infection and identified risk factors caused Campylobacteriosis in children with 51% and proposed that children aged less than 3 years at risk of *Campylobacter* infection if residing in a household which has puppies or chickens as a pets (Tenkate & Stafford, 2001)

4.2.2.1: Distribution of *Campylobacter* isolates according to age:

From the current study, out of 100 stool samples 11(11%) positive results showed a significant ($p \leq 0.05$) correlation between age and infection with Campylobacter spp., as the results show high infections in children under five years of age, and the prevalence rate decreases with increasing age table (2).

Table(4-5): Distribution of *Campylobacter* isolates according to age

Age rang (years)	Number of sample	Number of positive culture	Percentage %
2 month-1year	35	6	**17.14% a**
1-5 year	31	4	**12.9% ab**
5-10 year	34	1	**2.9% a**
Total	100	11	**11%**
Statistical analysis	**Chi square =3.49** ***p≤0.05***		

*Similar later mean significant while different latter mean no significant

There were significant difference between percentage of age groups where the first group (2month - 1year) has significant difference with third age group (5-10 year) while second group (1-5 year) has no significant differences with both first and third age groups as show in figure (4-7).

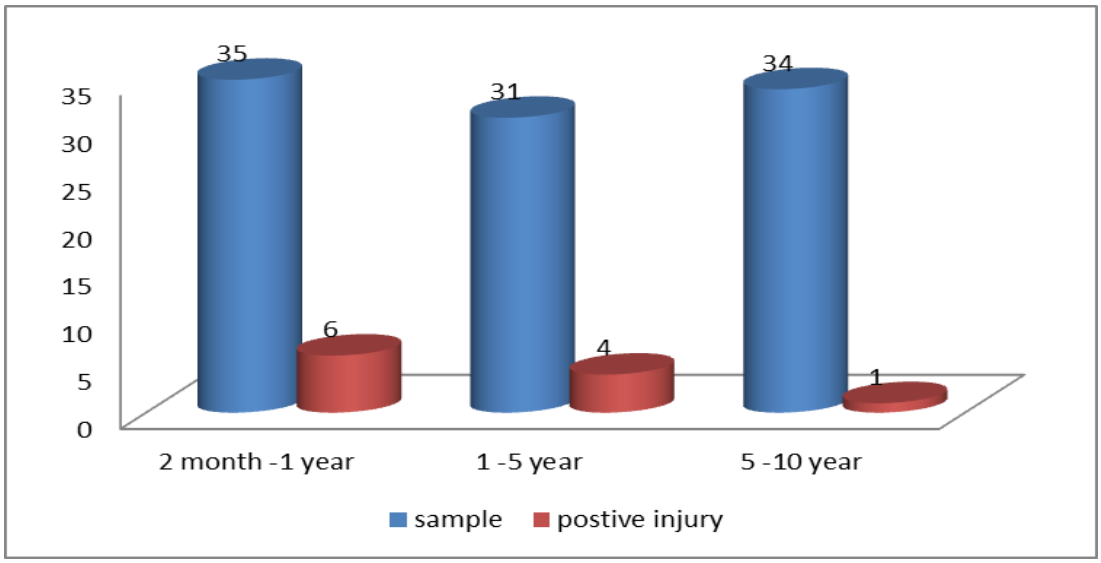


Figure (4-7): Distribution of *Campylobacter* isolates according to age.

The result of this study and other similar studies showed that the most effective age with *Campylobacter* is under five years that is due to their immunity is incomplete and the *Campylobacter* infection is considered as self-limited disease depended on the immune system (Nachamkin *et al*., 1998)(Singh *et al*., 2021).

These findings were agree with that founded by other researchers in some points in Brazil, (Upadhyay et al., 2019) mentioned that presenting significant association between the presence of the *C. jejuni* in children aged 0-12 months. In Québec /Canada ,the incidence rate in children less than 4 years was significantly higher than other ages that were significantly higher among people aged 15-34 years (Levesque *et al*., 2013). Furthermore, (WHO, 2017)demonstrated that *Campylobacter* infections in children under the age of 2 years are especially frequent, sometimes resulting in death. In developing countries where *Campylobacter* is endemic, infection is usually limited to children, with illness/infection ratios decreasing with age, suggesting that exposure in early life might lead to the development of protective immunity (Kaakoush *et al*., 2015)**,**(Ebrahimnezhad *et al*., 2020) and (Amin *et al.*, 2023)**.**

4.2.3. prevalence of *Campylobacter spp*. According to the seasonal preponderance

The prevalence of *Campylobacter spp.* infections in our study applied to certain months , the study period undertaken six months from October 2022 to the end of march 2023 as shown

Table (4-5): Prevalence of *Campylobacter spp.* According to the month

Month	No. of sample	Number of positive	Positive percentage
October	50	11	22%
November	50	9	18%
December	50	5	10%
January	50	3	6%
February	50	2	4%
March	50	8	16%
Total	300	38	12.6%
Statistical analysis	Chi secure = 11.253 P= 0.046 significant		

prevalence rate of (38) 12.6%. Data from(Table 4-5)can show the lowest rate of isolation in February 2 (4%), while the highest prevalence rate was in October 11 (22%), have significant difference, $p<0.05$ for most month as in figure (4-8)

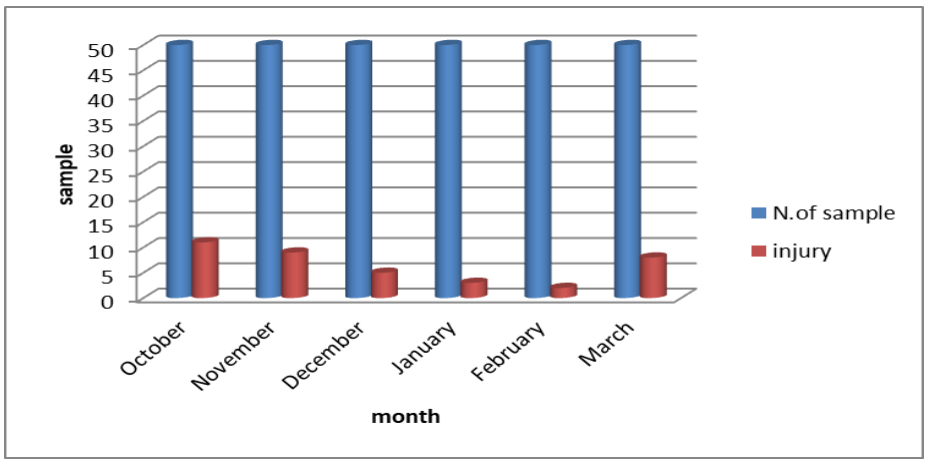


Figure (4-8): Prevalence of *Campylobacter spp.* According to the seasonal preponderance

In terms of time-based prevalence of *Campylobacter*, the highest occurrence was documented in early spring and early summer, while the lowest rate was found in winter.

These observations are in good line with a Nigerian study that reported that the number of campylobacteriosis peaks on summer season and then decline in winter season (Hlashwayo, Sigaúque, et al., 2020) .

Several studies had connected warm periods to high prevalence of *Campylobacter* (Zeleňáková *et al.,* 2021)Germany (Gölz *et al*., 2014)(Rosner *et al*., 2017), Egypt (Ahmed *et al*., 2015) , Lebanon (Ibrahim *et al.*, 2019)and Iraq (Abdulazeez, 2022). The underlying reason behind this seasonality is still unclear, but may indicate a possible association between temperature and *Campylobacter* survival and transmission of infection (Almashhadany, 2021)

The prevalence of Campylobacteriosis has marked seasonality in the temperate regions with peak risks in the summer while in tropical regions the seasonality was considerably less distinct (Rehman, 2022).

In Iraq , the incidence peak of Campylobacter infection in June to the end of August while in October ,November, December, January, February and in march ,with low positive detection (Al-Mawla *et al.*, 2008)in contrast with results of this study where in these months found different rates were found in except January .In Saudi Arabia, the seasonal prevalence peaked in October and November and various incidence rates in the rest of months with observation that seasonal distribution varies geographically (Abd, 2014)where the detection rates disagreed with results of present study.

4.3 Molecular identification

4.3.1 Molecular identification by 16S rRNA gene by PCR:

The molecular test used for identification and confirmation of *Campylobacter* as genus by PCR technique, PCR gave 38(12.6%) positive isolates showed to be *Campylobacter*. All examined isolates showed to be *Campylobacter* and were amplified 812 bp PCR products (Figure 4-9).

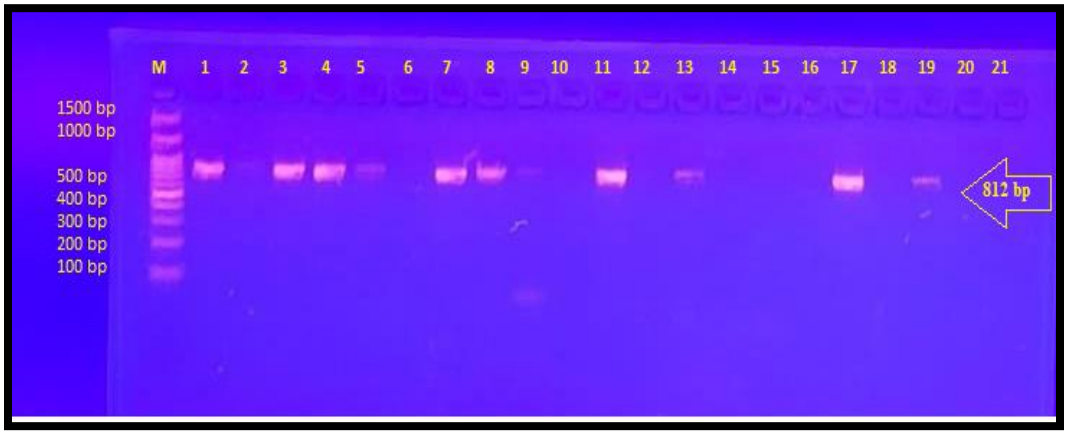


Figure (4-9): PCR results for detection of *Campylobacter* genus-specific *16S rRNA* gene. Lane M: (ladder)safe-green™ 1500 bp Opti-DNA Marker; Lanes numbered according to strain designation code. Lanes: 1, 3, 4, 5, 7, 8 ,11,17 ,19 shows positive results with *16S rRNA* gene (812 bp).

4.3.2 Typing by Sequence Method

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

Molecular identification of Campylobacter was applied using the 16S rRNA gene. The isolated samples amplicons were sent to MACROGEN® for sequencing using the Sanger sequencer. High quality sequences (reverse or forward) of nucleotides were identified and compared with archives available in the National Center for Biotechnology Information (NCBI) and classified

through the use of bioinformatics algorithms and programs specified for this type of analysis.

The results of the isolates showed a 100% match rate with the global isolates, and it was considered the first record of the isolates in the Genome Bank and was given special numbers as shown in Table (4-7).

Table No. (4-7) shows the numbers recorded in the Genome Bank of genetically diagnosed bacteria isolates a directly related display image in NCBI.

No.	Analysis	Accession number	Code	Source
1	*C. jejuni*	OQ253478.1	S1	MILK
2	*C.jejeni*	OQ318488.1	IQ4	Milk
3	*C. jejuni*	OQ283981.1	S3	Milk
4	*C.jejuni*	OQ287043.1	S4	Human
5	*C.Lari*	OQ318442.1	IQ1	Milk
6	*C. jejuni*	OQ253511.1	S7	Human
7	*C.Coli*	OQ318444.1	IQ2	Milk
8	*C –jejeni*	OQ284054.1	S10	Human
9	*C.jejuni*	OQ318512.1	IQ12	Human
10	*C. Coli*	OQ423040.1	IQ13	Milk
11	*C.jejuni*	OQ329425.1	IQ14	Human
12	*C.coli*	OQ330748.1	IQ15	Human
13	*C.jejnui*	OQ331024.1	IQ16	Human
14	*C.upsaliensis*	OQ338009.1	IQ 17	Human
15	*C. upsliensis*	OQ338139.1	1Q18	Human
16	*C.coli*	OQ338161.1	IQ19	Human

The result of isolation and diagnosis showed the presence and dominance of four genera of *campylobacter* (*C.Jejeni* , *C.coli* , *C.lari* and *C.upsaliense*) , which the higher percentage are *C.jejuni* (63%) and *C.coli* (26%) in milk and milk product as well as from human.

While the *C. lari* (5%) and *C. upsaliense* (5%) isolation results were very low in raw milk and human stool samples. As well as in table (4_8)

Table (4-8) type of *Campylobacter spp.*

Type	No.sample	*c.jejeni*	*c.coli*	*c.lari*	*c.upsalinses*
Milk	18	11 (61%)*	5(27%)	2(11%)	0
Milk product	9	6(66%)*	3(3%)	0	0
Human	11	7(63%)*	2(18%)	0	2(18%)
Total	38	24(63%)	10(26%)	2(5%)	2(5%)
Statistically analysis	P=0.267 Chi =7.62				

* Significant differences

The present results shown that *C. jejuni* was the most prevalent in relation to the number of samples, compared to *C.coli*. as show in figure (4-10)

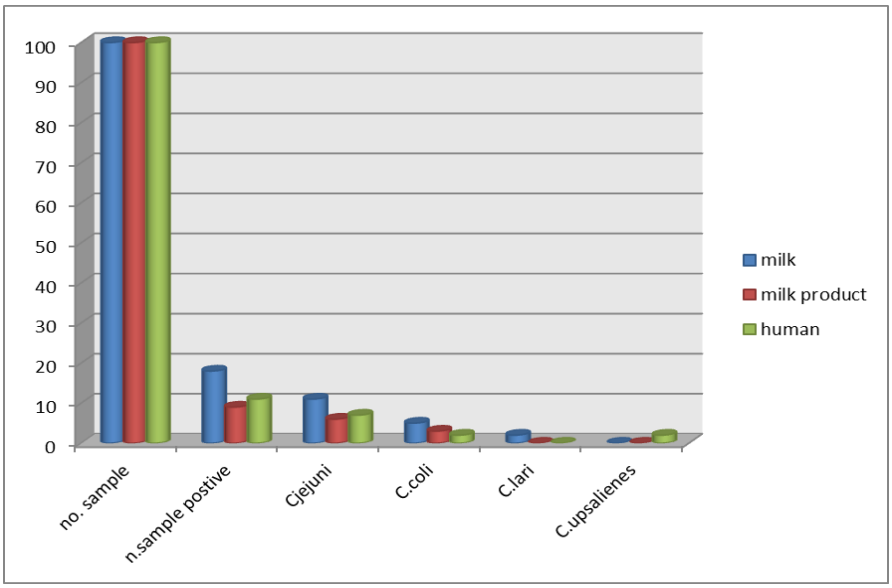


Figure (4-10): Percentage of Infection of *Campylobacter Spp*. In Karbala Governorate by Sequencing Analysis

The 16 *Campylobacter* strain isolates used in this study's bioinformatics analysis displayed very high similarity (approximately 99%) with It is common practice to identify between different *Campylobacter spp*. using 16S rRNA gene sequences as in (Lawton *et al*., 2018). Our findings provided significant evidence in favor of the approach to that mentioned and recommended by (Modi *et al.,* 2015). The findings did, in fact, concur with (Mahdi *et al*., 2022) *Campylobacter spp.* isolates previously found in Europe and America(Oakeson *et al*., 2018)

Typing by Sequence Method It is a high-resolution bacterial genotyping technique that has been useful in molecular studies (Dingle *et al.*, 2002), allowing the identification of two isolates with high efficiency in the production of gasteroenitierites. It 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. It was diagnosed using the polymerase

chain reaction (PCR) technique, depending on primers prepared for the purpose of molecular diagnosis (Mahdi *et al.*, 2022)**.** The 16 strains *of C. jejuni , C. coli ,C.upsaliense* , and *C.Lari* from this study's analysis of their phylogenetic relationships resulted in the creation of a phylogenetic tree.

A study of 16 Campylobacter strains recovered from milk and humans revealed that they came from various origins ,as show in Appendix(1)

The results showed that the registration of this bacteria is record in the global gene bank as shown in the registration information and the study of the affinity and similarity between the registered bacteria. To traditional diagnosis and genotype determination it is important in the classification of bacteria. The SSU region has been widely used in classification and molecular diagnosis due to its ease of amplification and its wide range of variability even in highly related species.

DNA sequencing to ensure the sequence of nucleotides and then compare it with other international strains, and the NCBI-BLAST program was used and gave accurate results by comparing it with international strains, and the genetic analysis program that used Molecular Evolutionary (BLAST) is an analysis application designed to compare similar gene sequences, evolutionary relationships, and the pattern of DNA and protein evolution (Kashoma et al., 2015)

The reason for 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 (Kaakoush *et al.*, 2015)

4.3.2.2Determination 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 using the NCBI program and compared with the data available in the National Center for Biotechnology Information (NCBI).)

Bacteria isolated from several genera and even species can be recognized using 16S rRNA genetic sequencing. The majority of *Campylobacter species* may be effectively distinguished using the 16S rRNA gene sequence. But according to this sequencing analysis, it was easy to separate *C. jejuni* from *C. coli* (Korczak et al., 2006)

The gene used in this work was compared to genes from position strains of *C. jejuni* (OQ253478.1) and C. coli (OQ318444.1) for phylogenetic analyses. The examination of the 16 sequenced isolates revealed that 10(26%) of the strains were *C. coli* and 24(63%) of the genomes matched those of *C. jejuni*.

According to table(4-7), the isolate (OQ253478.1) and (OQ318444.1) are genetically related and suggests that they may have originated from the same source. Although some of the other isolates were from the same farm, there is no strong similarity between them. This may be because the sources of infection were different for the other isolates.

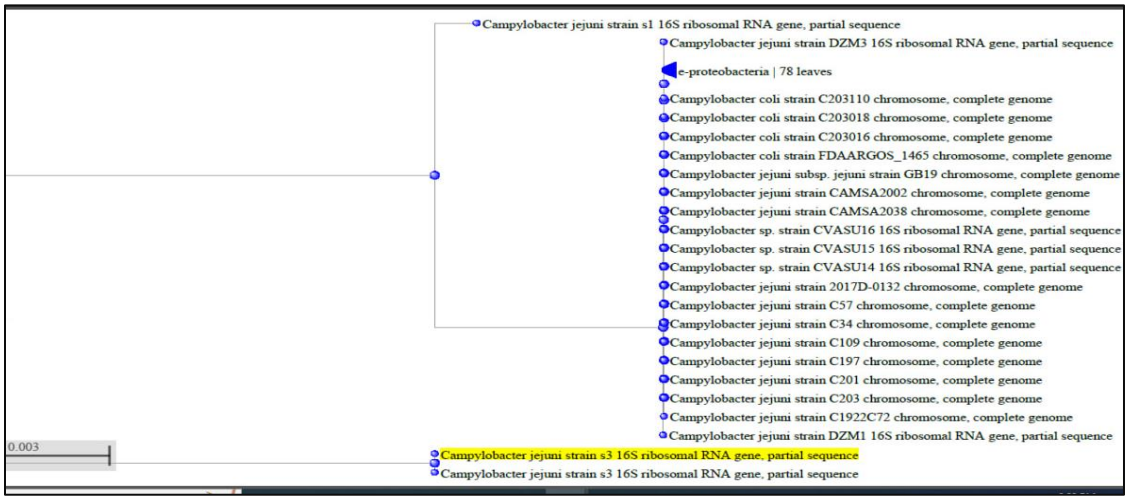


Figure (4-11): The genetic tree of *campylobacter jejuni* (marked in yellow), which was established based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method

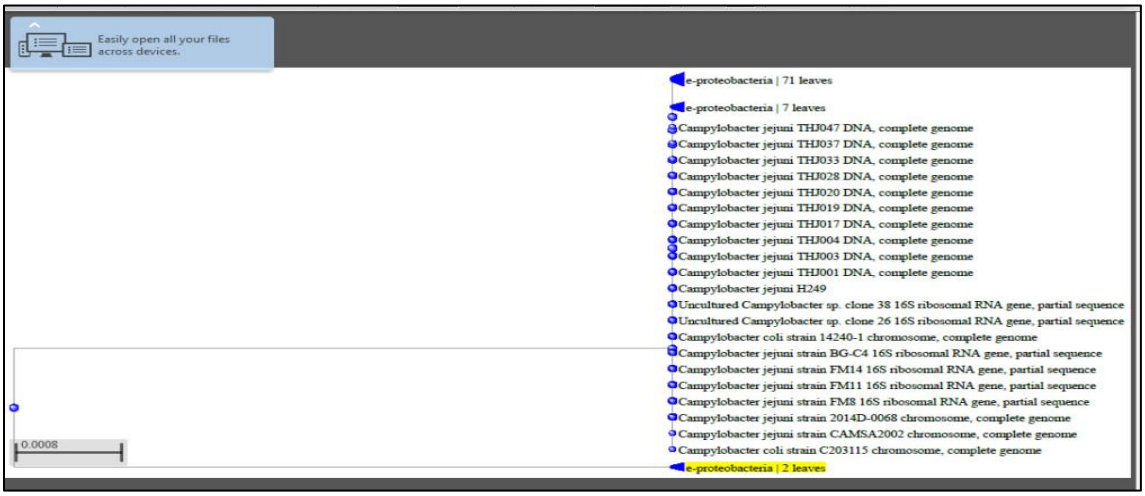


Figure (4-12): The genetic tree of campylobacter coli (marked in yellow), which was built based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method.

On the other hand, strains OQ338009.1 and OQ318442.1 were shown to be genetically related in (table 4-7) for partial sequencing analysis of the 16S rRNA gene of strains *campylobacter lari* and *campylobacter upsaliense.* The

investigation of the evolutionary relationships between the strains, however, revealed no similarities; this may be because the infections came from various origins.

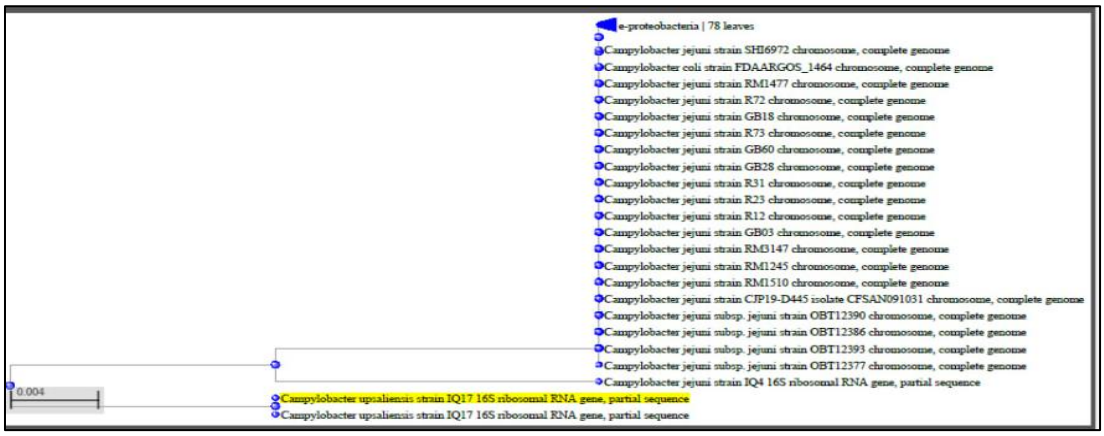


Figure (4-13): The genetic tree of *campylobacter upsalensis* (marked in yellow), which was built based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method

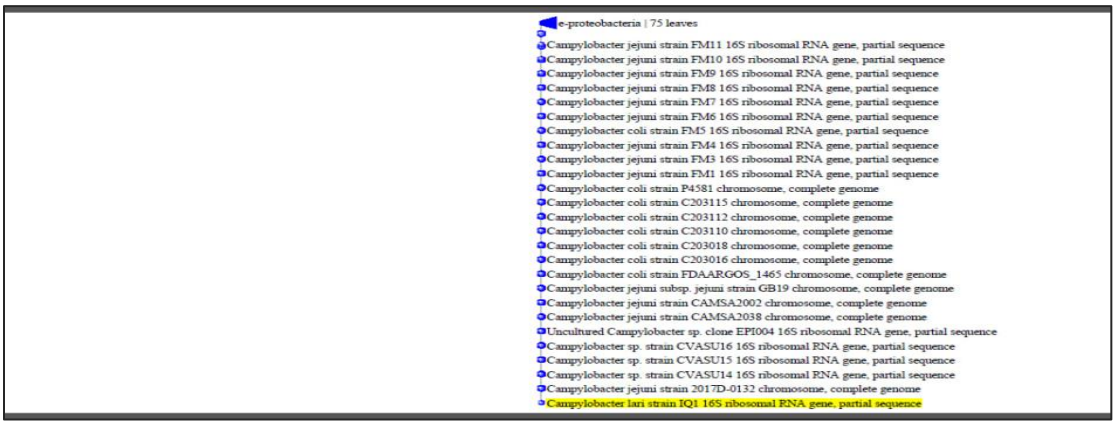


Figure (4-14) The genetic tree of campylobacter lari(marked in yellow), which was built based on the sequences of its nitrogenous bases in the ITS-rDNA region, in addition to the sequences of known global strains of the same pathogenic fungus obtained from the GenBank data warehouse. The genetic distances were calculated using the neighbor-joining method

In particular when isolates are of medical significance, 16S rRNA gene sequence analysis is particularly helpful for identifying bacteria at the genus and species level (Mahdi *et al*., 2022).

The two most common causes of foodborne bacterial diarrheal illness are *C. jejuni* and *C. coli*. Infections in humans are caused by *C. jejuni* 80–90% of the time and by *C. coli* only 7% of the time (Van Deun *et al.*, 2007)

The study's findings revealed a high prevalence of *C. jejuni*. comparable to C. coli in terms of frequency. (Rahimi, Sepehri, *et al*., 2013) showed that 76.4% of the samples were recognized as *C jejuni* and 23.6% *as C. coli*, which is consistent with our findings. Additionally, (Mahdi *et al.*, 2022) found that *C. jejuni* (78%) and *C. coli* (22%) were the two most common species in the samples.

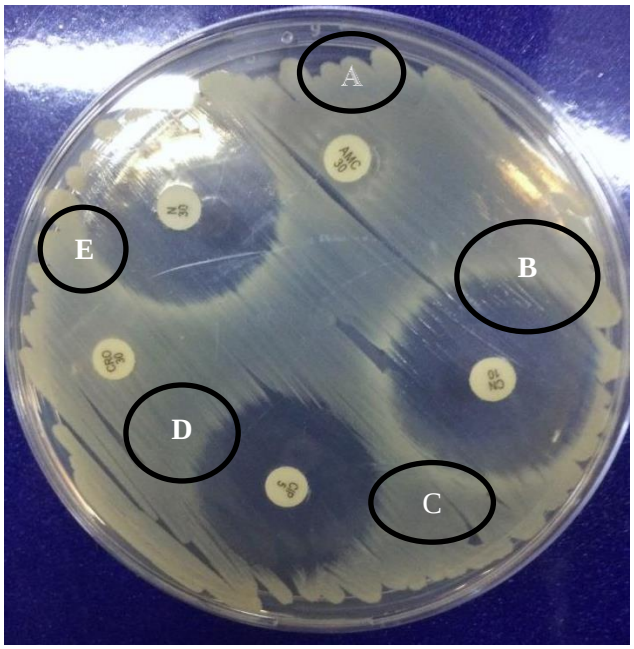
The outcomes also agree with those reported by (Issa *et al.*, 2018), who discovered that *Campylobacter jejuni* and 25% Campylobacter coli made up the majority of the thermophilic Campylobacter species isolated from chicken samples in Turkey.

(Adiguze*l et al.*, 2018) (Elmalı & Can, 2019)conducted a second investigation in Turkey and discovered that 14.2% were C. coli and 85.7% were *C. jejuni*. According to several studies (Barakat *et al*., 2020)**;**(Mahdi *et al.*, 2022) (Abdulazeez, 2022)**;** *C. jejuni* is more common than *C. coli.*

According to the results of previous studies that were presented to regions of neighboring countries such as Iran and Turkey, our results agree with them mostly. In Iraq, milk , and industry materials are imported from Iran and Turkey, as well as milk product which may be a sufficient reason for the similarity of insulation rates in Iraq with neighboring countries.

4. Evaluation of antibiotic susceptibility test.

All 38 isolates were tested for their susceptibility to 12 antimicrobial drugs and classified as resistant, and susceptible, as in figure (4-11)



A:AMC(amoxicillin)

B:CN(cephalexin)

C:CIP(ciprofloxacin)

D:CRO (ceftriaxone)

E:N(nalicdixic acid)

Figure (4.15):This figure demonstrates the extreme resistance of some *Campylobacter spp*. isolates according to the antibiotic susceptibility test.

Modified Kirby-Bauer disk diffusion method was employed to evaluate the susceptibility of Campylobacter isolates to twelve antibiotics according to CLSI guidelines (CLSI, 2020)**.**The Enterobacteriaceae breakpoints published by CLSI were used to interpret the inhibition zones diameters around antibiotic disks. The tested antibiotics (Mast diagnostics, UK) were: Azithromycin, ciprofloxacin, Ceftriaxone, nalidixic acid, gentamicin Penicillin, tetercycline, Cephalexin, Imipenem , Ampiciline, amoxciline, and streptomycine.

4.4.1 Antibiotic resistance to *Campylobacter jejuni* & *Campylobacter upsaliense*

The prevalence of susceptibility to each antibiotic tested is presented in Table (4-9) ,from positive 38 (12.5%) sample ,which isolated 24 *Campylobacter jejuni* & 2 *campylobacter upsaliense* as resistant and sensitive pattern.

Table 4-9: Relationship of Antimicrobial Agent and Zone of inhibition *Campylobacter jejuni*

Number	Antibiotic	Concentration	Zone of inhibition No-sample=24			
			Sensitive	Intermediate	Resistance	P-value
1.	Azithromycin	15 mg	**8(33%)**	**1(4%)**	**15(63%)**	**0.0021 ****
2.	Ciprofloxacin	5 mg	**13(54%)**	**2(8%)**	**9(38%)**	**0.0199 ***
3.	Ceftriaxone	30 mg	**5(21%)**	**4(17%)**	**15(63%)**	**0.0093 ****
4.	Nalicdixic acid	30 mg	**7(29%)**	**7(29%)**	**12(50%)**	**0.0271 ***
5.	Gentamicin	10 mg	**12(50%)**	**3 (13%)**	**9(38%)**	**0.070 NS**
6.	Penicillin	10 mg	**3(13%)**	**7(29%)**	**14(58%)**	**0.0199 ***
7.	Cephalexin	10 mg	**4(17%)**	**3(13%)**	**17(70%)**	**0.0005 ****
8.	Tetracycline	30 mg	**3(13%)**	**1(4%)**	**19(79%)**	**0.0001 ****
9.	Imipenem	10 mg	**20(83%)**	**2(8.3%)**	**2(8.3%)**	**0.0001 ****
10.	Ampiciline	10 mg	**11(45%)**	**3(12.5)**	**10(42%)**	**0.091 NS**
11.	Amoxicillin	25 mg	**10(42%)**	**1(4%)**	**13(54%)**	**0.0073 ****
12.	Streptomycin	10 mg	**2(8%)**	**4(17%)**	**18(75%)**	**0.0001 ****
P-value	---	---	**0.0001 ****	**0.046 ***	**0.0074 ****	---
*** (P≤0.05), ** (P≤0.01).**						

Campylobacter jejuni showed in our study high sensitive to imipenem (83%), gentamycin (50%), and ciprofloxacin (54%) while total resistance was recorded to ampicillin, streptomycin, and tetracycline .

Table (4-10): Relationship of Antimicrobial Agent and Zone of inhibition *Campylobacter upsaliense*

Number	Antibiotic	Concentration	Zone of inhibition No-sample=2			
			Sensitive	Intermediate	Resistance	P-value
1.	Azithromycin	15 mg	**2(100%)**	**0**	**1(50%)**	**0.003 ** **
2.	ciprofloxacin	5 mg	**2(100%)**	**0**	**0**	**0.021 ***
3.	Ceftriaxone	30 mg	**1(50%)**	**0**	**1(50%)**	**0.0032 ****
4.	Nalicdixic acid	30 mg	**1(50%)**	**1(50%)**	**0**	**0.0271 ***
5.	Gentamicin	10 mg	**2(100%)**	**0**	**0**	**0.065NS**
6.	Penicillin	10 mg	**1(50%)**	**0**	**1(50%)**	**0.0188 ***
7.	cephalexin	10 mg	**1(50%)**	**1(50%)**	**0**	**0.0076** ***
8.	Tetracycline	30 mg	**1(50%)**	**0**	**1(50%)**	**0.0002 ****
9.	Imipenem	10 mg	**2(100%)**	**0**	**0**	**0.0032 ****
10.	Ampiciline	10 mg	**0**	**0**	**2(100%)**	**0.065 NS**
11.	Amoxicillin	25 mg	**10(42%)**	**1(4%)**	**13(54%)**	**0.0053** ***
12.	Streptomycin	10 mg	**0**	**0**	**2(100%)**	**0.0022 ****
P-value	---	---	**0.0001 ****	**0.046 ***	**0.0074 ****	**---**
*** (P≤0.05), ** (P≤0.01).**						

Campylobacter upsaliense showed in our study high sensitive to imipenem (100%), gentamycin (100%), and azithromycin (100%) while total resistance was recorded to ampicillin, streptomycin, and tetracycline There are wide

variations in the antibiotic resistance patterns of *Campylobacter* isolates in published literature from different countries around the globe.

the current study's finding of ciprofloxacin resistance is in good accord with levels previously reported in Iran (30.77% and 34.4%) (Wysok *et al.*, 2011)((Maktabi *et al*., 2019)**;** (Rahimi, Ameri, *et al.*, 2013). Although higher than that reported from India (Modi *et al.*, 2015)the high sensitivity to gentamicin and streptomycin revealed in this study is still lower than that. A recent Tanzanian research of cattle carcasses and raw milk samples is likewise in good accord with the resistance levels to ampicillin, erythromycin, and gentamicin (Kashoma *et al*., 2016)

Strains prospering in various ecological niches, geographic regions, the usage of antibiotics, and horizontal gene transfer of resistance determinants all have an impact on these variances in resistance phenotypes (Aksomaitiene *et al.*, 2019)**;** (Zhang *et al.*, 2020)**.**

4.4.2 Antibiotic with *Campylobacter coli* and *Campylobacter lari.*

The prevalence of susceptibility to each antibiotic tested is presented in table(4-9), From the total positive 38 (12.5%), which isolated 10 *Campylobacter coli and 2 Campylobacter lari as* resistant and sensitive patterns.

Table (4-11): Relationship of Antimicrobial Agent and Zone of inhibition *Campylobacter coli*

No	Antimicrobial Agent	Concentration	Zone of inhibition Campylobacter coli no .sample=10			
			S	I	R	P-value
1	Azithromycin	15 mg	(80%)	0	2(20 %)	0.010 **
2	Ciprofloxacin	5 mg	5(50%)	2(20%)	3(30 %)	0.492 NS
3	Ceftriaxone	30 mg	4(40%)	2(20%)	4(40 %)	0.667 NS
4	Nalidixic acid	30 mg	4(40%)	0	6(60 %)	0.050 *
5	Gentamicin	10 mg	9(90%)	1(10%)	0	0.0014 **
6	Penicillin	10 mg	3(30%)	3(30%)	4(40 %)	0.903 NS
7	Cephalexin	10 mg	7(70%)	1(10%)	2(20 %)	0.0436 *
8	Tetracycline	5 mg	8(80%)	1(10%)	1(10 %)	0.0071 **
9	Imipenem	10 mcg	10(100%)	0	0	0.0003 **
10	Ampicillin	10 mg	3(30%)	2(20%)	7(7 %)0	0.011 **
11	Amoxciline	25mg	4(40%)	1(10%)	5(50 %)	0.713 NS
12	Streptomycine	10 mg	5(50%)	3(30%)	2(20 %)	0.492 NS
P-value	---	---	0.031 *	0.091 NS	0.044 *	---
	* (P≤0.05), ** (P≤0.01)					

Which showed significant differences ($p<0.01$) ($P\leq0.05$),The current result show high sensitive to imipenem is 2(100%) and gentamicin 2(100%)As in table (4_12)

Table (4-12): Relationship of Antimicrobial Agent and Zone of inhibition *Campylobacter lari*

No	Antimicrobial Agent	Concentration	Zone of inhibition *Campylobacter lari* no .sample=2			
			S	I	R	P-value
1	Azithromycin	15 mg	1(50%)	0	1(50%)	0.02**
2	Ciprofloxacin	5 mg	1(50%)	1(50%)	0	0.321NS
3	Ceftriaxone	30 mg	0	1(50%)	1(50%)	0.541NS
4	Nalidixic acid	30 mg	1(50%)	0	1(50%)	0.020*
5	Gentamicin	10 mg	2(100%)	0	0	0.0013 **
6	Penicillin	10 mg	1(50%)	1(50%)	0	0.882 NS
7	cephalexin	10 mg	1(50%)	0	1(50%)	0.0351*
8	Tetracycline	5 mg	1(50%)	0	1(50%)	0.0021**
9	Imipenem	10 mg	2(100%)	0	0	0.0005 **
10	Ampicillin	10 mg	0	0	2(100%)	0.044 **
11	Amoxciline	25mg	0	1(50%)	1(50%)	0.651NS
12	Streptomycine	10 mg	0	1(50%)	1(50%)	0.543 NS
P-value	---	---	0.031 *	0.091 NS	0.044 *	---
	* ($P\leq0.05$), ** ($P\leq0.01$).					

There are wide variations in antibiotic resistance pattern of *Campylobacter* isolates from various nations are documented in published works.. Strains that thrive in various ecological niches, geographic regions, the usage of antibiotics, and horizontal gene transfer of resistance determinants all have an impact on these differences in resistance phenotypes (Almashhadany, 2021)

The *Campylobacter* isolates used in our investigation have a significant level of tetracycline and azithromycin resistance as well. *Campylobacter* from raw milk had previously been found to be more resistant to this antimicrobial agent in Poland in Similar data regarding the isolation of *Campylobacter* species from milk that were highly resistant to ciprofloxacin and tetracycline (greater than 85%) were reported by (Modi *et al.*, 2015).In contrast, (Kashoma *et al.*, 2016) reported that only 9.3% and 11.8% (depending on the method) of *Campylobacter* isolated from milk samples and beef carcasses, respectively, exhibiting resistance to ciprofloxacin .Similar rates for ciprofloxacin resistance were detected in the US An outbreak of fluoroquinolone-resistant *Campylobacter* infections associated with raw milk consumption in United States of America was recently described by (Resistance, 2019)

The resistance of *Campylobacter coli & campylobacter lari campylobacter* to the antibiotic Gentamycin was somewhat low and may be due to The sensitivity of his strains to their production of aminoglycoside-modifying enzymes was consistent with what was found by (EMILIA, n.d.). And his group in 2000, in addition to the fact that many studies recorded that Gentamycin is effective in the treatment of *Campylobacter* as the causative

agent of the intestinal tract. (Velàzquez *et al.*, 1995)**;** (Velázquez-Ordoñez *et al.*, 2019);(Admasie *et al*., 2023).

Our result corresponds with the report of (Abbasi *et al.*, 2019)who also observed high phenotypic *Campylobacter* isolates resistant to tetracycline, ciprofloxacin and erythromycin. The report of (Nisar *et al*., 2017) also showed high *Campylobacter* resistant to gentamycin (25.6%) and our finding is also in line with their report. Another study of Premarathne et al also observed high *Campylobacter* resistant to ampicillin and this report correspond with our result.(Premarathne *et al*., 2017)**,**(Igwaran & Okoh, 2020)**.**

The reason for the non-existent resistance of this bacterium to the two antibiotics, impeniem and gent, may gentamycin This is due to the lack of prior use of these two antibiotics by patients with diarrhea under study And resorting to other available and prescribed antibiotics to treat cases of diarrhea. The potential dangers to consumers highlight the need for effective control measures, particularly the careful use of antibiotics to reduce the spread of *Campylobacter* that is antibiotic-resistant.

Chapter Five

Conclusions

And

Recommendations

5.1 Conclusions:

1.This is the first study reporting prevalence and estimating impact of some risk factors of Campylobacter infection in human, milk and milk products, and use molecular test to confirm the diagnosis.

2. According to the sequence registration of local isolates, four *Campylobacter spp. appeared (C. jejuni, C. coli,C.coli ,C.lari* and *C. upsaliense).*

3. The present data showed an increase prevalence rate in October and November.

4.The infection rates were inversely recorded with age and were higher at younger ages.

5.The antibiotic susceptibility test used to investigate campylobacter resistance to antibiotics.

5.2 Recommendations:

1- Conducting an experimental study on the harmful effects of these isolated strains

2- More surveys are needed throughout the year to know the full epidemiological picture of Campylobacter in milk.

3- Raising awareness, consumer education and raise the level of hygiene of milk and dairy products to prevent microorganisms that cause foodborne illnesses.

4- Caution in the use of antibiotics, preferably after conducting a sensitivity test, to prevent the emergence of resistant bacterial strains.

5.Random antibiotics usage should be restricted to minimize public health hazards of spreading multi-drug resistance pathogens.

6. The use of modern sterilization methods and packaging that prevent the occurrence of food borne disease food .

Chapter six
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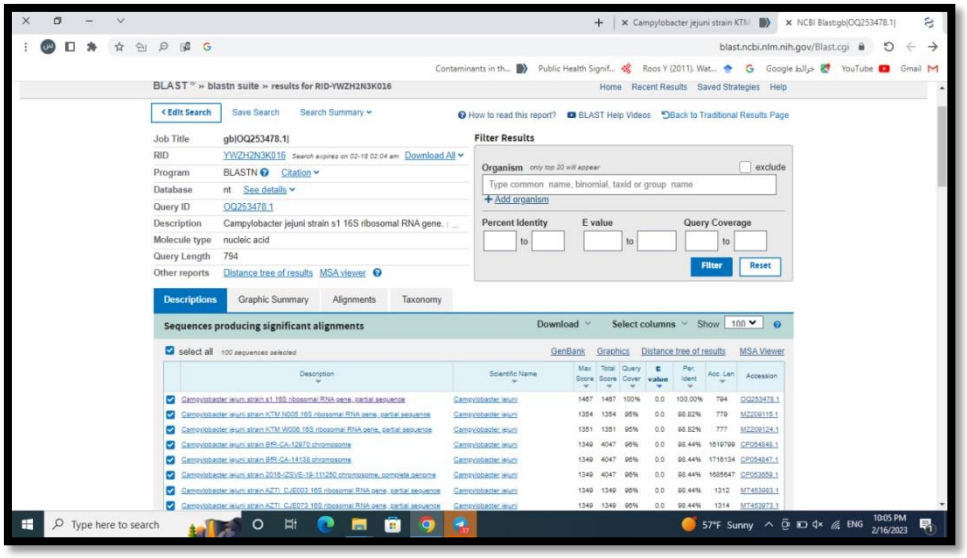
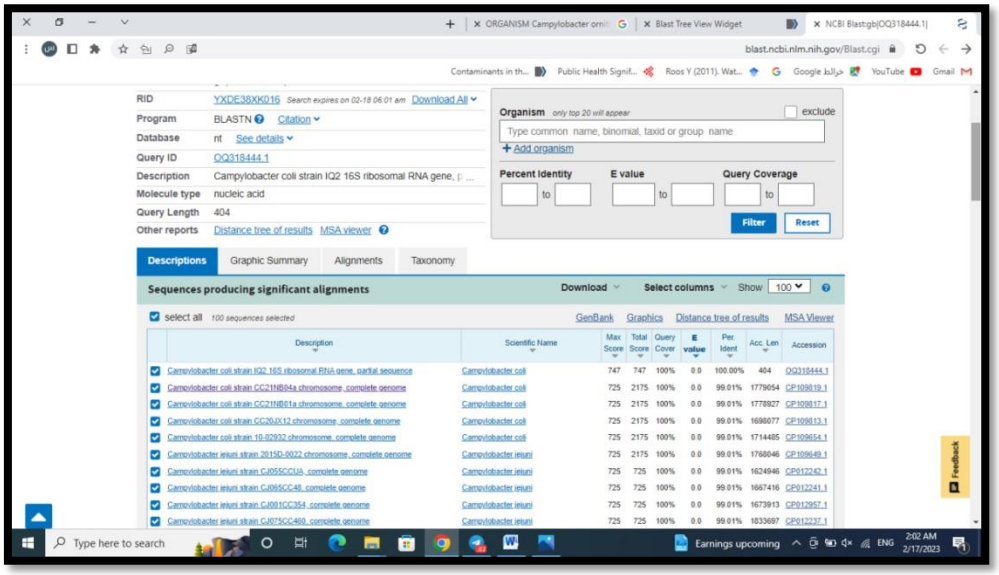
Appendix

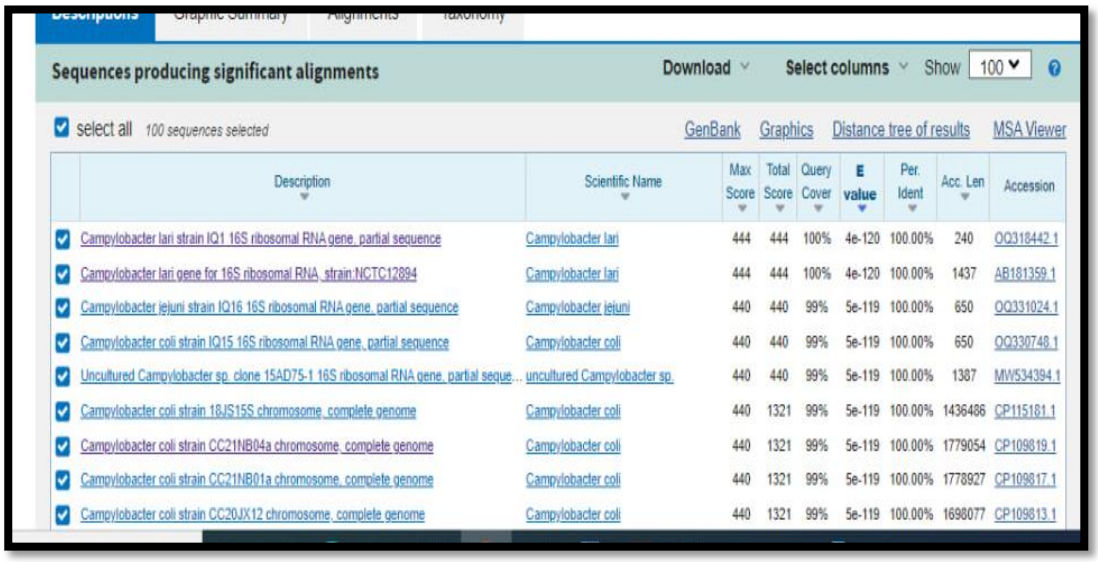
Appendix

Campylobacter jejuni isolates match global isolates in the GenBank





matches *campylobacter coli* with global isolates in the GenBank

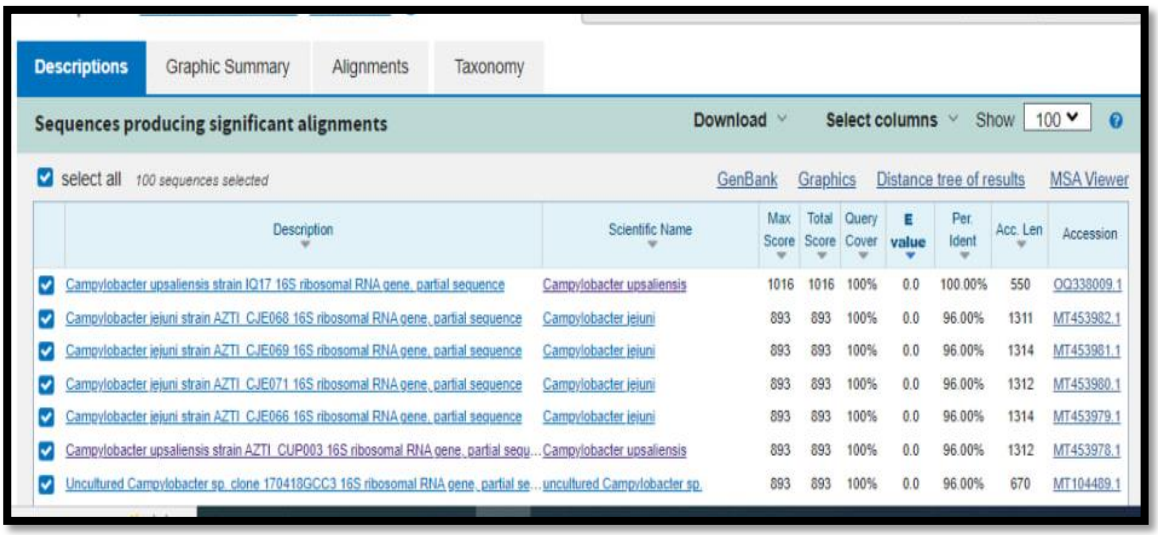


Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Campylobacter lari strain IQ1 16S ribosomal RNA gene, partial sequence	Campylobacter lari	444	444	100%	4e-120	100.00%	240	OQ318442.1
Campylobacter lari gene for 16S ribosomal RNA, strain:NCTC12894	Campylobacter lari	444	444	100%	4e-120	100.00%	1437	AB181359.1
Campylobacter jejuni strain IQ16 16S ribosomal RNA gene, partial sequence	Campylobacter jejuni	440	440	99%	5e-119	100.00%	650	OQ331024.1
Campylobacter coli strain IQ15 16S ribosomal RNA gene, partial sequence	Campylobacter coli	440	440	99%	5e-119	100.00%	650	OQ330748.1
Uncultured Campylobacter sp. clone 15AD75-1 16S ribosomal RNA gene, partial seque...	uncultured Campylobacter sp.	440	440	99%	5e-119	100.00%	1387	MW534394.1
Campylobacter coli strain 18JS15S chromosome, complete genome	Campylobacter coli	440	1321	99%	5e-119	100.00%	1436486	CP115181.1
Campylobacter coli strain CC21NB04a chromosome, complete genome	Campylobacter coli	440	1321	99%	5e-119	100.00%	1779054	CP109819.1
Campylobacter coli strain CC21NB01a chromosome, complete genome	Campylobacter coli	440	1321	99%	5e-119	100.00%	1778927	CP109817.1
Campylobacter coli strain CC20JX12 chromosome, complete genome	Campylobacter coli	440	1321	99%	5e-119	100.00%	1698077	CP109813.1

matches *campylobacter lari* with global isolates in the GenBank



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Campylobacter upsaliensis strain IQ17 16S ribosomal RNA gene, partial sequence	Campylobacter upsaliensis	1016	1016	100%	0.0	100.00%	550	OQ338009.1
Campylobacter jejuni strain AZTI_CJE068 16S ribosomal RNA gene, partial sequence	Campylobacter jejuni	893	893	100%	0.0	96.00%	1311	MT453982.1
Campylobacter jejuni strain AZTI_CJE069 16S ribosomal RNA gene, partial sequence	Campylobacter jejuni	893	893	100%	0.0	96.00%	1314	MT453981.1
Campylobacter jejuni strain AZTI_CJE071 16S ribosomal RNA gene, partial sequence	Campylobacter jejuni	893	893	100%	0.0	96.00%	1312	MT453980.1
Campylobacter jejuni strain AZTI_CJE066 16S ribosomal RNA gene, partial sequence	Campylobacter jejuni	893	893	100%	0.0	96.00%	1314	MT453979.1
Campylobacter upsaliensis strain AZTI_CUP003 16S ribosomal RNA gene, partial sequ...	Campylobacter upsaliensis	893	893	100%	0.0	96.00%	1312	MT453978.1
Uncultured Campylobacter sp. clone 170418GCC3 16S ribosomal RNA gene, partial se...	uncultured Campylobacter sp.	893	893	100%	0.0	96.00%	670	MT104489.1

***Campylobacter Upsalensis* with global isolates in the GenBank**

الخلاصة

بكتريا *Campylobacter spp.* هو أحد مسببات الأمراض الرئيسية التي تنتقل عن طريق الغذاء والتي توجد بشكل متزايد في جميع أنحاء العالم وتنتقل إلى الإنسان عن طريق اللحوم والحليب الخام ومنتجات الألبان .

هدفت هذه الدراسة إلى تحديد مدى انتشار بكتيريا *Campylobacter spp.* في عينات من براز الإنسان والحليب الخام ومنتجات الألبان في مدينة كربلاء المقدسة والكشف عن حساسية هذه العزلات للمضادات الحيوية، حيث بدأت الدراسة الحالية من تشرين الأول 2022 إلى نهاية آذار 2023.

حيث اخذت (200) عينة جمعت بطريقة معقمة : 100 عينة من الحليب الخام و 100 عينة من منتجات الألبان من المتاجر والمزارع المحلية المختلفة الموزعة في محافظة كربلاء بالإضافة إلى 100 عينة من براز الأنسان يتراوح عمر الأطفال بين شهرين إلى 10 سنوات حيث كانو يعانون من الإسهال والحمى وآلام البطن وتم جمع العينات من مستشفى الأطفال التعليمي ومستشفى الحسين التعليمي. في محافظة كربلاء.

وتم زراعة العينات مباشرة على الوسائط المخصبة والانتقائية .التي تظهر مستعمرات مخاطية صغيرة ذات لون رمادي نموذجي وبعضها يجب أن يكون رمادي اللون، مرتفع قليلا، رطب، وكثيرا ما ينتج مستعمرات منفصلة، مسطحة مع حواف غير منتظمة، وغير انحلالية في 24-48 ساعة هي خصائص العطيفة .النيابة .مستعمرات معزولة على الوسط الانتقائي لقاعدة Campylobacter agar.

واستخدمت طريقة تفاعل البلمرة PCR المتسلسل، تم التأكد من أن 38 عزلة من أصل 300عينة (12.6%) هي ..*Campylobacter spp.*

ومن النتائج التي أظهرت تم إخضاع ستة عشر عزلة للتسلسل للكشف عن أنواع بكتريا *Campylobacter spp.* وتم تسجيل أربعة أنواع منها *(C.lari, C.coli, C.jejuni, C.upsaliense)*

وفقا لحساسية البكتريا للمضادات الحيوية من خلال فحص الانتشار القرصي، أظهر اختبار الحساسية للمضادات الميكروبية لـ *C.Jejuni* و *C.upsaliense* حساسية كاملة ل

Gentimycin Impenim Ciproflaxcin بينما أظهرت المقاومة الكاملة لحمض Cephalexin Streptomycin Tetracycline من ناحية أخرى، كانت بكتيريا *C.Coli* و *C.lari* حساسة كلياً ل Gentamycin و Impenim و Azithromycin بينما لوحظت أيضاً مقاومة ل Ampicillin و.Amoxicillin

نمط المقاومة المكتشف في هذه الدراسة مثير للقلق للغاية بسبب عدم الحساسية للمضادات الحيوية المذكورة سابقًا، والتي تستخدم بشكل روتيني كأدوية مفضلة لعلاج داء العطيفة .وارتبط الموسم الدافئ بزيادة انتشار بكتيريا العطيفة في الحليب الخام .ونظرًا للمخاطر المحتملة على الصحة العامة، فإن مستويات الانتشار والمقاومة هذه تستحق تحقيقًا إضافيًا وعلاجات مناسبة...

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب البيطري

عزل وتوصيف اجناس بكتريا *Campylobacter* من الانسان والحليب الخام ومنتجاته باستخدام تقنية الفحص الجزيئي في محافظة كربلاء

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في فرع الصحة العامة

بواسطة

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