

INCIDENCE OF CAMPYLOBACTER SPECIES IN MILK AND SOME MILK PRODUCTS IN ALEXANDRIA CITY

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ABSTRACT

A total of 200 samples including 50 samples of raw milk, 50 samples of Kareish cheese, 50 samples of Damietta cheese and 50 samples ice-cream, all were collected randomly from Alexandria markets and street vendors and examined bacteriologically for the detection and identification of existing Campylobacter microorganisms. The result recorded in this investigation declared that Campylobacter could be isolated from raw milk, Kareish cheese, Damietta cheese and ice-cream in percentages of 10, 14, 4 and 8% respectively.

Campylobacter jejuni which is pathogenic for human as it is responsible for food poisoning and other gastrointestinal symptoms associated with bloody diarrhoea was isolated from raw milk in 3 cases (6%), from Kareish cheese in 3 cases (6%), from ice-cream in 4 isolates (8%) and could not be isolated from Damietta cheese. The presence of Campylobacter in this study reflect the lack of hygiene supervision and poorly cleaned and sanitized dairy farm equipments and processing plant equipments. Moreover, Campylobacter jejuni isolated in this study was tested for susceptibility to 11 antimicrobial drugs. All isolates were highly sensitive and susceptible to Naladixic acid and Norfloxacin but show high resistance to Tetracyclin and Cephalothin and some extend resistance to Erythromycin, Flumequine and Pencillin. The public health hazard to Campylobacter jejuni and other Campylobacter species and the sanitary measures for improving milk quality beside proper handling and distribution of milk and milk products must be done under the most possible hygienic measures.

INTRODUCTION

Campylobacter has been associated with gastroenteritis in human of all ages, systemic infections, abortion as well as prenatal diseases (Skirrow, 1977 and Franco, 1988). Many authors incriminated the group of *Campylobacter* species as a cause of outbreaks of food poisoning (Bryan, 1992 and Corry and Hinton, 1997). Exposure to *Campylobacter* was greatest in developing countries where standard of hygiene was poor (Hamdy and Shahat, 1995).

Campylobacter may be found as a normal flora in intestine of both wild and domesticated animals specially those used for food production (Penner, 1988). However, the main source of *Campylobacter* infection is probably raw milk and milk products which are the most commonly implicated vehicles in food-borne outbreaks of *Campylobacter* enteritis (Richter et al., 1992 and Bean et al., 1996). Contamination of milk can occur by direct excretion from an asymptomatic cow with mastitis (Hutchinson et al., 1985) or through fecal contamination during milking from cattle infected or colonized with the organisms (Waterman et al., 1984 and Humphery and Beckett 1987). Also post pasteurization contamination of milk and dairy products have been found responsible for *Campylobacter* outbreaks (White, 1986). *Campylobacter* comprises many species which are now considered as bacterial agents of clinical importance in humans. The most frequently identified human pathogenic species of *Campylobacter* are *Campylobacter jejuni* and *Campylobacter coli* which are closely related and their infection appear to share many clinical and epidemiologic characteristic in addition to *Campylobacter laridis* and these species were constituted about more than 90% of the isolates in human infection. *Campylobacter jejuni* has become the most commonly reported cause of food borne enteritis in people in the world (Skirrow, 1990 and Butzler and Oosterom, 1991).

Campylobacter can produce different types of toxins such as enterotoxin, cytolethal distending toxin and cytolethal rounding toxin. *Campylobacter* enteritis has been associated with some complications such as arthritis, recurrent colitis and haemolytic uremic syndrome (Allos, 1997). *Campylobacter* is a Gram-negative cylindrical, curved, and motile rod. It is a microaerophilic organism, as it requires a reduced levels of oxygen for growth. It is relatively fragile and sensitive to environmental stresses such as 20% oxygen, drying, heating, disinfectants and acidic conditions. Because of its microaerophilic characteristics, the organism requires 5% oxygen and 10% carbon dioxide for optimal growth (Betty *et al.*, 1998). The fast majority of outbreaks of Campylobacteriosis have been associated with consumption of unpasteurized or inadequately pasteurized cow's milk in Newzeland, Scotland, Switzerland and England (Hutchinson *et al.*, 1985; Hudson *et al.*, 1990) as well as dairy products (Barrett, 1986).

In Egypt, acute diarrhoea of presumed infectious origin is responsible for more than 50% of deaths for those under two years of age (Ewyda, 1990). Therefore, *Campylobacter* species still continue to be highly important human pathogens and there is an increase of *Campylobacters* particularly *Campylobacter jejuni* as a health risk affecting both human and animals, and because of the involvement of milk and milk products in human *Campylobacter* enteritis, in addition, increasing antimicrobial resistance of *Campylobacter* infection cases was a recognized problem. The present work was designed to study the following:

- Occurrence of *Campylobacter* in milk and some milk products.
- Identification of the isolated organisms.
- Sensitivity patterns of the isolated *Campylobacter jejuni*.

MATERIAL AND METHODS

A total of 200 samples including raw milk (50), Kariesh cheese (50), Damietta cheese (50) and ice-cream (50) were collected randomly from Alexandria markets and street vendors. All samples were transferred to the laboratory in an ice box without delay for bacteriological examination in relation to *Campylobacter* microorganisms.

Preparation of samples:

All milk samples were tested by peroxidase test to exclude heat-treated milk according to *Lampert (1975)*. Kareish cheese, Damietta cheese and ice-cream samples were thoroughly mashed and mixed in sterile mortar according to the technique recommended by *APHA (1992)*.

Pre-Enrichment:

1 ml or gm of each prepared sample were incubated into *Campylobacter* enrichment broth and *Brucella* broth. Each broth containing 5% horse blood, Skirrow *campylobacter* selective supplement and Skirrow *campylobacter* growth supplement. The inoculated tubes were incubated at 42°C for 24 hour in atmosphere of 5% oxygen , 10% carbon dioxide and 85% nitrogen using an anaerobic jar and *campylobacter* gas generation kits (*Oxoid, 1990*).

Selective plating:

Incubated broth cultures were then streaked onto Skirrow medium (*Skirrow, 1977*) containing 5-7% horse blood and *campylobacter* selective supplement (*Oxoid*). The plates were incubated at 42°C for 48 hours in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen in standard anaerobic jar.

The bacterial isolates were purified and identified by using colonial morphological and biochemical characteristics according to *Koneman et al., 1988; Skirrow, 1990 and Quinn et al., 2002*).

In-Vitro drug sensitivity test:

Different types of antibacterial sensitivity discs were used in the sensitivity tests to determine the sensitivity and resistance of *Campylobacter jejuni*. The drug discs were obtained from Oxoid. The technique was carried out using disc diffusion method according to *Bopp et al. (1985)*. Three colonies of *Campylobacter* organism were inoculated into tubes containing 5 ml Muller Hinton broth (Oxoid), then incubated for eight hours under reduced oxygen tension at 37C° until the turbidity exceeded that of the standard McFerland 0.5 barium sulphate tube (0.5 ml of 1.175% barium chloride hydrate to 99.5 ml of 1% sulphuric acid). The turbidity was adjusted to match that of the McFerland 0.5 barium sulphate standard tube by adding sterile saline solution. The suspension was then inoculated evenly on 150 mm Muller-Hinton agar plates supplemented with 5% defibrinated sheep blood. Drug discs were placed on the surface of agar plate in a radial pattern with the lowest concentration toward the center. The plates were incubated for 72 hours at 37C° under the microaerophilic conditions and the inhibitory zone diameters were measured.

RESULTS

Bacteriological examination of 200 samples including 50 samples of raw milk, 50 samples of Kariesh cheese, 50 samples of Damietta cheese and 50 samples of ice-cream revealed the isolation of *Campylobacter* species in percentages of 10, 14, 4 and 8% respectively Table (1).

As shown in Table (2), *Campylobacter jejuni* could be isolated from the examined milk samples, Kareish cheese samples, Damietta cheese samples and ice-cream samples in 3(6%), 3(6%), 0, 0% and 4(8%) respectively. The biochemical characteristics of *Campylobacter jejuni* are illustrated in Table (3).

In-vitro antimicrobial sensitivity test Table (4), showed that *Campylobacter jejuni* isolates were sensitive to Nalidixic acid and Norfloxacin, (100%) followed by Neomycin, Gentamycin and Amoxicillin (80%), while 60% only sensitive to Ampicillin. On the other hand, all isolates of *Campylobacter jejuni* were resistant to Tetracycline and Cephalothin (100%) and 80% of isolates were resistant to Erythromycin, penicillin and Flumequine.

Table (1): Incidence of *Campylobacter* species in the examined samples of milk and milk products.

Kind of samples	No. of samples examined	Positive samples	
		No.	%
Milk	50	5	10
Kareish cheese	50	7	14
Damietta cheese	50	2	4
Ice-cream	50	4	8

Table (2): Incidence of *Campylobacter jejuni* in the examined samples of milk and milk products.

Kind of samples	No. of samples examined	Positive samples	
		No.	%
Milk	50	3	6
Kareish cheese	50	3	6
Damietta cheese	50	0	0
Ice-cream	50	4	8

Table (3): Biochemical characteristics of *Campylobacter jejuni* in the examined samples of milk and milk and milk products.

Test	Reactions (Result)
Catalase test	+ve
Oxidase	+ve
H ₂ S of lead acetate	+ve
H ₂ S of TSI	-ve
Nitrate reduction	+ve
Hippurate hydrolysis	+ve
Glycin (1%) tolerance test	+ve
Sodium chloride (3.5%) tolerance test	-ve
Growth at 25°C	-ve
37°C	+ve
42°C	+ve

Table (4): Antimicrobial sensitivity of *Campylobacter jejuni* isolated from milk and milk product samples (n=10).

Types of antibiotics	Degree of sensitivity	% of sensitivity
Ampicillin 10mg	+	60%
Amoxicillin 30mg	++	80%
Cephalothin 30mg	Resistant	0
Erythromycin 15mg	Resistant 80%	20%
Flumequine 30mg	Resistant 80%	20%
Gentamycin 10mg	++	80%
Naladixic acid 30mg	+++	100%
Neomycin 30mg	++	80%
Norfloxacin 10mg	+++	100%
Penicillin 10 IU	Resistant 80%	20%
Tetracyclin 30mg	Resistant	0

DISCUSSION

Food born bacterial gastrointestinal infections are important causes of morbidity and mortality world-wide, and despite successful control programs in some developed countries, these infections continue to have a major impact on public health economy (*Varnam and Evans 1991*). *Campylobacter* species are one of the most reported pathogens incriminated in gastrointestinal illness in domestic animals and humans throughout the world (*Atabay and Corry, 1997*).

Recently, *Campylobacter jejuni* is considered the most commonly reported cause of food born enteritis in people in the world *Skirrow, 1990 and Butzler and Oosteron, 1991*).

Incidence of *Campylobacter* microorganisms in raw milk samples:

Results recorded in Table (1) showed that 5 isolates (10%) of the examined milk samples were positive for *Campylobacter*, from which 3 isolates were identified by biochemical reaction as *Campylobacter jejuni* (6%), Table (2). These results are nearly in agreement with those obtained by *Abd-El-Hady (1993) and Nagah et al., (2007)*. Lower percentages were stated by *Inokova and Ivanova (1996), Hudson et al. (1990) and Duzgun et al. (2000)*. However, several investigators failed to detect *Campylobacter* species in the examined milk samples such as *Mouffok and Lebres (1992) and Federighi et al., (1999)*.

The variation in these results may be attributed to different factors, including level of contamination and methods of isolation, variety of enrichment broth systems, height sensitivity of the organisms to normal atmospheric concentration of oxygen and to adverse conditions resulting from acid development in raw milk that represent stress factor on the organism resulting in failure of cultural trials even from contaminated samples. *(Ray and Johnson, 1984)*. The presence of *Campylobacter* species in the examined raw milk samples may be attributed to poor hygienic conditions under which raw milk is produced and handled, contamination of milk during or after milking is probably of faecal origin, however, improper washing and treatment of the udder with suitable disinfectant may result of high level of contamination. Furthermore, naturally occurring *Campylobacter* mastitis and contaminated water supply may act as a source of milk contamination *(Mentzing, 1981)*.

Incidence of Campylobacter in Kareish cheese:

In the present study *Campylobacter* could be isolated from 7 out of 50 kareish cheese samples examined (14%) Table (1). The 3 strains of *Campylobacter* isolates were identified as *Campylobacter jejuni* as presented in Table (2).

Lower result was reported by *Naghah et al., (2007)*. *Campylobacter* species failed to be recovered from kareish cheese samples examined by *Abdelhady (1993)* and *Federighi et al., (1999)*. The result of this investigation indicates that kareish cheese collected from local market and street vendors in Alexandria was of poor sanitary quality and could cause considerable risk to human health.

Incidence of Campylobacter in Damietta cheese:

As recorded in Table (1) it is apparent that out of 50 examined samples of Damietta cheese two samples were positive for *Campylobacter* species (4%). This result nearly similar with that obtained by *El-Nokrashy et al. (1997)* and *Nagah et al., (2007)*. Several investigators failed to isolate *Campylobacter* species from Damietta cheese (*Bachmann, 1994* and *Federighi et al., 1999*). Lower incidence or absence of the isolation of the organism in a such product may be due to its fragile nature and its sensitivity to adverse conditions of acid development as *Campylobacters* are inactivated at pH 4.5 (*Doyle and Roman, 1981*).

Incidence of Campylobacter in Ice-cream:

The data presented in Table (1) showed that out of 50 examined samples of ice-cream 4 samples (8%) contained *Campylobacter* species which could be identified as *Campylobacter jejuni* Table (2). Several investigators failed to isolate *Campylobacter* species from ice-cream

(*Ray and Johnson 1984 and Abd El-Hady 1993*), other investigator could be isolated *Campylobacter* species from ice-cream (*Nagah, 2007*). The lower or absence incidence of the organism in such products could be attributed to its sensitivity to conditions of freezing which stressed *Campylobacter* organisms and resulted in failure to recover the organism from contaminated frozen food.

In vitro, drug sensitivity test was done against isolated strains of *Campylobacter jejuni* using a panel of 11 antibiotic Table (4). The most effective antibiotics for all isolates of *Campylobacter jejuni* are Naladixic acid and Norfloxacin, while it was resistant to Cephalothin and Tetracycline. Moreover 80% of the isolates were moderately sensitive to Amoxicillin, Gentamycin and Neomycin. These results are nearly similar to those reported by *Mouffak and Lebres (1992)* and *Diker (1987)*.

It is of value to note that, consumption of raw milk and milk products contaminated with *Campylobacter* species has a potential hazard.

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مدى تواجد ميكروبات الكامبيلوباكتز فى اللبن وبعض منتجات الألبان فى مدينة الإسكندرية

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معهد بحوث صحة الحيوان - فرع الإسكندرية

يعتبر اللبن الخام ومنتجاته من أكثر الأغذية استهلاكاً نظراً لقيمتها الغذائية. إلا أنه يكون مسبباً لكثير من الأمراض عن طريق التلوث بالميكروبات سواء من الماشية المريضة أو أثناء الإنتاج والنقل. والتصنيع والتداول. ومن هذه الميكروبات ميكروب الكامبيلوباكتز الذى يؤدى إلى مغمص قولونى مصحوب بأسهال مدمم. وقد اشتملت الدراسة على فحص عدد 200 عينة من الألبان ومنتجاته عبارة عن 50 عينة لبن خام، 50 عينة جبن قريش، 50 عينة جبن أبيض طرى دمياطى و 50 عينة آيس كريم. وقد تم تجميع هذه العينات من محلات وأسواق مدينة الإسكندرية وقد أسفر العزل البكتريولوجى عن تواجد ميكروب الكامبيلوباكتز بنسبة 10% فى عينات اللبن وبنسبة 14% فى عينات الجبن القريش وبنسبة 4% فى عينات الجبن الأبيض دمياطى وبنسبة 8% فى عينات الآيس كريم.

وفى اللبن الخام تم تصنيف ميكروب الكامبيلوباكتز جوجناى فى ثلاث عينات وبنسبة 6% بينما تم عزل وتصنيف نفس الميكروب فى عدد 3 عينات من الجبن القريش وبنسبة 6% أيضاً، بينما لم يتم عزله وتصنيفه فى الجبن دمياطى، كما تم عزله وتصنيفه فى جميع العينات الايجابية فى الآيس كريم وهى أربع عينات. وبدراسة اختبار الحساسية لميكروب الكامبيلوباكتز جوجناى وجد أن أكثر العقاقير تأثير عليه وبنسبة 100% فى النلادكسك أسيد والنورفلوكساسين بينما يقاوم الميكروب المضاد الحيوى التتراسيكلين والفليموكوين.

هذا وقد تمت دراسة ومناقشة ووضع الضوابط والشروط الصحية والاقتصادية والمقترحات

الواجب إتباعها للمحافظة على اللبن ومنتجاته من التلوث بهذه الميكروبات.