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# STUDIES ON SOME FOOD POISONING BACTERIA IN RAW MILK SOLD IN SHARKIA GOVERNORATE

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**ABSTRACT**

One hundred raw farm bulk milk samples were collected from Sharkia Governorate. A survey was conducted to determine the incidence of food poisoning bacteria in raw milk and experiments were carried to determine antibiotic sensitivity of *Campylobacter jejuni* & *Yersinia enterocolitica* and *proteolytic* & *lipolytic* activities of isolated Pseudomonas spp. Out of the 100 raw farm bulk milk samples tested 1 (1%), 1 (1%) and 2 (2.0%) were found to contain, *Campylobacter jejuni* against Karmali, Columbia and modified Preston agar, respectively. Serotyping of *Campylobacter jejuni* isolated from the examined raw milk samples resulted in two strains were belonged to *Campylobacter jejuni* serotype 2 while only one was *Campylobacter jejuni* serotype 1. Antibiotic sensitivity revealed that all the tested *Campylobacter jejuni* isolates were 100% sensitive to Genitamycin and Chloramphenicol while none were sensitive to Cefoperazone and Ampicillin. *Yersinia enterocolitica* and *Yersinia krestensenii* could be detected in 7% and 4% respectively when cultured on bile oxalate sorbose enrichment broth, while using Yersinia enrichment broth (YEB) 7.33% and 4% of milk samples were positive for *Yersinia enterocolitica* and *Yersinia krestensenii* respectively. 50% of the examined samples were contaminated with *Pseudomonas* spp. The high level of contamination was $0.5 \times 10^8$ ; the low level was $2.3\times 10^2$ and the mean value was $1.8 \times 10^4 \pm 0.5 \times 10^4$. Also, it is found that out of the examined 100 raw bulk milk samples 2 (2%) were positive for *E. coli*.

***Key words:*** *Food Poisoning, Raw milk, Antibiotics*

## INTRODUCTION

Food-borne illness was principally associated with five well-recognized pathogens. These include: *Staphylococcus aureus*, Salmonella spp., *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus*. However, each year the etiological agents responsible for food-borne diseases were not identified for more than 50% of outbreaks. Many reasons may explain this frequent inability to identify organisms, including the fact that many outbreaks were caused by previously unrecognized pathogens or by known pathogens not previously recognized as agents of food-borne illness. Within the past 10 to 15 years, several other pathogens had been identified as important causes of food-borne diseases including *Campylobacter jejuni*, *Yersinia enterocolitica* and *E. coli* 0157:H7 (Doyle, 1992).

Milk and milk products have frequently implicated in the transmission of human pathogens, including Salmonella spp., *Campylobacter jejuni* and *Yersinia enterocolitica*. Because proper pasteurization kills these pathogens, most milk-borne outbreaks of human illness have been associated with raw or inadequately pasteurized milk or with milk contaminated after pasteurization (Bryan, l983).

More than a decade ago; *Campylobacter jejuni* was considered as a pathogen primarily of veterinarian significance. Within the last decade, *Campylobacter jejuni* had been recognized as gastroenteritis Pathogen.

*Yersinia enterocolitica* has been isolated from many animal species, with most isolates being a virulent for humans. Exception is swine, they are the principal reservoir for virulent strains, which are often isolated from oral cavity (tongue and tonsils) of apparently healthy animals. Outbreaks caused by agents have included chocolate and pasteurized milk (Anonymous, 1977 and Tacket *et al.,* 1984) More recently, *E. coli* serotype O157:H7 has recently emerged as a significant food-borne pathogen, causing hemorrhagic colitis in human and hemorrhagic uremic syndrome (Eley, 1996).

Therefore the present study was undertaken to investigate the incelence of prevalence of *Campylobacter jejuni*; Yersenia spp.*;* Pseudomonas

spp. and *E. coli* in raw milk sold in Sharkia Governorate.

## MATERIALS and METHODS

**Sampling:**
One hundred random raw milk samples were collected from different dairy farms in Sharkia Governorate.

500 ml of milk proved to be raw by storch test (FDA 1998) were collected in a sterile capped bottle. All samples were placed into an insulated ice-box and transferred to the laboratory within one hour of sampling. The samples were held in refrigerator (4 – 7°C) until examination within 12 hours.

**1 - Isolation and identification of Campylobacter jejuni according to (FDA 1998):**
**Pre-enrichment:**
The pH value of the raw milk was adjusted using pH test paper (pH 6-8 range) if the pH is below 7.6, sterile 1-2 N NaOH was added and gently adjust to 7.5 ± 0.2.

50ml milk were centrifuged at 20000-x g for 40 minutes. Supernatant was discarded and pellet (not fat layer) was dissolved in 10 ml enrichment broth (Bolton broth) supplemented with vial each of FBP and Bolton broth selective supplement.

The pellet was transferred to 90 ml enrichment broth in screw-capped bottle. The enrichment broth was incubated at 42°C for 48 hrs.

**Plating on selective media:**
After appropriate enrichment, loopfuls from each liquid culture were streaked onto the following media:

(1) Columbia agar base, supplemented with Blaser-Wang supplement and 5-7% laked horse blood (Hundson *et al.,* 1999).

(2) Campylobacter agar base (Karmali) supplemented with campylobacter selective supplement (Lovett *et al.,* 1983).

(3) Campylobacter blood-free selective medium (modified CCDA-Preston) supplemented with CCDA selective supplement (Federighi *et al.,* 1999).

Inoculated plates were incubated at 42°C/48 hrs in case of Columbia agar base and Karmalli, and at 37°C/48 hrs for modified CCDA Preston, microaerobically (in an atmosphere consists of approximately 5-6% Oxygen, 10% Carbon dioxide and 84-85% Nitrogen). This is achieved by using campygen CN25 in conjugation with 2.5-liter capacity anaerobic jar.

Antibiotic sensitivity of campylobacter from examined raw milk samples according to Wells *et al.* (1987).

**2- Isolation and Identification of Yersinia in milk according Thisted and Danielsson (2005).**
**3- Isolation and identification Psudomonaus, according to Peters *et al.* (2006).**
**4- Isolation and identification *E. coli* according to Crochshang 1975.**

## RESULTS

**Table 1:** Prevalence of *Campylobacter jejuni* in the examined raw farm bulk milk samples

| No. of samples | Types of media | | | | | |
|---|---|---|---|---|---|---|
| | Karmali | | Columbia agar base | | Modified Preston (CCDA) | |
| 100 | Positive samples | | Positive samples | | Positive samples | |
| | No. | % | No. | % | No. | % |
| | 1 | 1 | 1 | 1 | 2 | 2 |

**Table 2:** Serotyping of isolated *Campylobacter jejuni* from the examined raw farm bulk milk samples

| Serotypes | Types of media | | | | | |
|---|---|---|---|---|---|---|
| | Karmali | | Columbia agar base | | Modified Preston (CCDA) | |
| | No. | % | No. | % | No. | % |
| Cj2* | 1 | 1 | 1 | 1 | 1 | 1 |
| Cj1** | - | - | - | | 1 | 1 |
| Total | 1 | 1 | 1 | 1 | 2 | 2 |

*CJ2 = *Campylobacter jejuni* 2 **CJ1 = *Campylobacter jejuni* 1

**Table 3:** Antibiotic sensitivity *of Campylobacter jejuni* isolated from examined raw farm bulk milk samples

| *Types of Antibiotics* | *Campylobacter jejuni isolates* | | | | | | | *% of sensitivity* |
|---|---|---|---|---|---|---|---|---|
| | **CJ2** | **CJ2** | **CJ2** | **CJ2** | **CJ2** | **CJ1** | **CJ1** | |
| Gentamycin 10 ugm | S | S | S | S | S | $S^+$ | $S^+$ | 100 |
| Tetracycline10 ugm | S | S | S | MS | S | MS | MS | 100 |
| Erythromycin 10 ugm | R | R | R | R | R | R | MS | 14.29 |
| Cefoperazone 30 ugm | R | R | R | R | R | R | R | 0 |
| Clindamycin 2 ugm | S | S | S | S | S | MS | MS | 100 |
| Ampicillin 10 ugm | $R^+$ | $R^+$ | R | R | $R^+$ | R | R | 0 |
| Nalidixic acid 30 ugm | S | S | S | S | S | S | S | 100 |
| Chloramphenicol 30 ugm | $S^+$ | $S^+$ | $S^+$ | $S^+$ | S | S | S | 100 |

**Table 4:** Prevalence of *Yersinia spp*. in examined raw farm bulk milk samples

| *Total No.* | *Yersinia enterocolitica* | | | | *Yersinia kristensenii* | | | |
|---|---|---|---|---|---|---|---|---|
| | **BOS** | | **YEB** | | **BOS** | | **YEB** | |
| | **Positive sample** | | **Positive sample** | | **Positive sample** | | **Positive sample** | |
| 100 | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| | 7 | 7 | 6 | 6 | 5 | 5 | 4 | 4 |

**Table 5:** Statical analytical results of Pseudomonas spp. in examined raw milk.

| *No samples* | *Positive samples* | | *Minimum* | *Maximum* | *Mean* | *± SEM* |
|---|---|---|---|---|---|---|
| | *No* | *%* | | | | |
| 100 | 50 | 50 | $2.3\times10^2$ | $5\times10^6$ | $1.8\times10^4$ | $0.54\times10^4$ |

**Table 6:** Frequency distribution of Pseudomonas spp. isolated from the examined raw farm bulk milk samples

| *Pseudomonas strains* | *No. of positive Samples* | *% of isolates in relation to No. of positive samples* |
|---|---|---|
| *Pseudomonas aeruginosa* | 20 | 16.6 |
| *Pseudomonas cepacia* | 17 | 14 |
| *Pseudomonas fluorescens* | 50 | 50 |
| *Pseudomonas maltophilia* | 20 | 16.6 |
| *Pseudomonas pickitti* | 13 | 10.8 |
| **Total** | 120 | 100 |

**Table 7:** Prevalence of *E. coli* O157: H7 in the examined raw farm bulk milk samples

| **No of samples** | ***E. coli* O157: H7** | |
|---|---|---|
| 100 | **No. of positive samples** | **%** |
| | 2 | 2 |

## DISCUSSION

The results given in Table 1 show that out of examined 100 raw farm milk samples, 1 (1%) 1 (1%) and 2 (2.0%) were positive for *Campylobacter jejuni* on Karmali agar, Columbia agar base and modified Preston, respectively. These findings are in agreement with those reported by Lovett *et al.* (1983) and Franco (1988), slightly higher than the results obtained by Hudson *et al.* (1999). El-Nokrashy *et al.* (1997) could isolate *Campylobacter jejuni* from raw milk samples with higher percentages. While Mouffok and Lebres (1992) and Federighi *et al.* (1999) could not isolate *Campylobacter jejuni* from raw milk.

Serological identification of isolated Campylobacter are listed in Table 2 which show that the one *Campylobacter jejuni* strain recorded on Karmali agar medium belonged to *Campylobacter jejuni* serotype 2 and one *Campylobacter jejuni* isolate obtained on Columbia agar base was assigned as *Campylobacter jejuni* serotype 2. Modified Preston agar medium recovered 2 *Campylobacter jejuni* strains, which serologically assigned as *Campylobacter jejuni* serotype 2 and *Campylobacter jejuni* serotype 1.

Similar findings were reported by El-Nokrashy *et al.* (1997). Penner and Heniessy (1980) mentioned that most of the tested *Campylobacter jejuni* isolates were serologically identified as *Campylobacter jejuni* serotype 1 and *Campylobacter jejuni* serotype 2 while Fitzgerald *et al.* (2001) serotyped 9 *Campylobacter jejuni* strains using Somatic O typing and found that all isolates were Cj19.

Table 3 summarizes the antibiotic sensitivity of isolated *Campylobacter jejuni* from examined raw bulk milk samples. All the tested isolates (10%) were sensitive to Gentamycin 10 ugm, Tetracycline l0 ugm, Clindamycin 2 ugm, while non of the isolates were sensitive to Cefoperazorie 30 ugm, Ampicillin 10 ugm. Out of the tested isolates 14.29% were sensitive to erythromycin 10 ugm. These results are in agreement with those reported by Wells *et al.* (1987) while nearly similar findings were reported by Karmali *et al.* (1981) and Palmgren *et al.* (1997). It has been receded that *Campylobacter jejuni* survive better in food at refrigeration temperature than at room temperature. The pathogen may remain viable in sterile milk at 4$^{o}$C for up to 22 days, whereas at 25$^{o}$C no viable organism could be detected after 3 days (Blaser *et al.,* 1980; Rollins and Colwell, 1986 and *Curtis et al.,* 1995).

Sufficient pasteurization at 62.8$^{o}$C for 30 minutes inactivate the pathogens even when milk contains large numbers of the bacterium (Aoust *et al.,* 1988).

Prevalence of *Yersinia enterocolitica* in farm bulk milk samples presented in Table 4 revealed that of 100 tested farm bulk milk samples 7 (7%) were found to be contaminated with *Yersinia enterocplitica* when cultured on Bile-oxalate-sorbose (BOS) compared with 6 (6%) when cultured on Yersinia enrichment broth (YEB). The results given in Table 4 show that out of 100 raw milk samples tested 5 (5%) and 4 (4%) were positive for *Yersinia kristensenii* using BOS and YEB, respectively. These findings are in agreement with those obtained by Saad and Moustafa (1989); A1i (1990) and Cotton and White (1991). Slightly higher incidences were recorded by Schiemann and Toma (1978) and Franzin *et al.* (1984).

Prevalence of Pseudomonas spp. in examined raw bulk samples are listed in Table 5, which shows that 50 /100 (50%) of tested samples contained Pseudomonas spp. Nearly similar incidences were reported by Katona (1981) and Ahmed (1995) while Otte *et al.* (1978) and Kalogridou Vasiliadou and Manalkidis (l984) recorded slightly lower values.

The high level of Pseudomonas contamination was $0.5 \times 10^{6}$; the low level was $2.3 \times 10$ and the mean value was $1.8 \times 10^{4} \pm 0.54 \times 10^{4}$. These findings are in agreement with that reported by Ahmed (1995) while lower levels were reported by Bruzynska *et al.* (1974). Desmasures, and GueGnen (l997) examined 34 refrigerated milk samples and found that pseudomonas count was $5.8 \times 10^{2}$.

Out of 60 Pseudomonas spp., *Pseudomonas fluorescens* was found to be comprise up to 50% of the total isolates. *Pseudomonas maltophilia*, *Pseudomonas aeruginosa*, *Pseudomonas pickiti* and *Pseudomonas cepacia* were comprising 16.6%, 16.6%, 10.8% and 14.1% respectively (Table 6). These findings are in agreement with that reported by Ahmed (1995) while, Juffs (1973) & Rashed and Buddary (1981) could report higher values. Lower incidences were declared by Uraz and Citak (1998).

The results given in Table 8 revealed, that out of 100 examined raw farm bulk milk samples, only 2 (2%) contained *E. coli* O157:H7. These finding are in agreement with those reported by Wells *et al.* (1987) while lower incidence was reported by Steele *et al.* (1997), while Gooding and Choudary (1997) and Palmgren *et al.* (1997) could not detect *E. coli* O157:H7 in any of examined raw milk samples. It was reported that most of hemorrhagic colitis outbreaks resulted form consumption of under cooked minced beef or raw milk and dairy cattle have been identified as a reservoir of E. coli O157:H7 (Blanco *et al.,* 1996).

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## دراسات عن بعض البكتريا المسببة للتسمم الغذائي في اللبن الخام المباع في محافظة الشرقية

***أحمد عبد الخالق السيد ، السيد السعيد مسعود ، مني طلعت رسلان***

تم تجميع ١٠٠ عينة من ألبان المزارع المباعة في محافظة الشرقية وذلك لعمل مسح لبعض البكتريا المسببة للتسمم الغذائي وذلك لمعرفة مدى وجود كل من الكامبيلوباكترجوجناي واليارسينيا انتيروكولوتيكا والسيدوموناس والأيشرشياكولاي. وقد وجد أن عينة واحدة بنسبة ١ % تحتوي علي الكامبيلوباكتر جوجناي باستخدام الكرامل آجار وعينة واحدة موجبة باستخدام الكولومني آجار وعدد ٢ عينة موجبة بنسبة ٢% باستخدام موديفيد برستون آجار. وبعمل تصنيف سيرولوجي للكامبيلوباكتر جوجوناي المعزولة وجد أنها عترتان هما C.J2 و C-J1. وكانت عترات الكامبيلوباكتر المعزولة من اللبن حساسة للجينتاميسين بنسبة ١٠٠% وأيضاً الكلورمفينكول ولكنها غير حساسة للسيفوبيروزون والأمبيسيللين. وكانت نسبة العزل ٧% يارسينيا أنتروتيكا و ٤% اليارسينيا كرستينس عندما زرعت على أوكسلات سوربوز بينما عندما زرعت على يارسينيا بروس (YEB) كانت النسبة ٧.٣٣ يارسينيا انتولوتيكا و ٤% يارسينيا كرستينس. وكانت نسبة العزل من السيدوموناس ٥٠% من العينات وكان أعلى مستوى من التلوث ٠.٥ × ١٠^٨ بينما كان أقل مستوى من التلوث ٢ × ١٠^٢ وكان المتوسط ١.٨ × ١٠^٤ ± ٠.٥×١٠^٤ وتواجدت الأيشريشياكولاي بنسبة ٢% من العينات وصنفت سيرولوجياً على أنها O157.