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OCCURRENCE OF *YERSINIA ENTEROCOLITICA* AND AEROMONAS HYDROPHILA IN PASTEURIZED MILK IN SOHAG CITY

(With 3 Tables)

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تواجد ميكروب اليارسينيا أنتيروكوليتـكا والأيروموناس هيدروفيلا في اللبن المبستر في مدينة سوهاج

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أجريت هذه الدراسة لمحاولة عزل ميكروبـــى اليارســينيا أنتيروكوليتكـــا والأيروموناس هيدروفيلا من خمسين عينة من اللبن المبستر جمعت عشوائياً من أكشاك الألبـــان والســوبر ماركت بمدينة سوهاج وقد أسفرت النتائج على أن ميكروب اليارسينيا أنتيروكوليتكـــا أمكـــن عزلها بنسبة ٤%. أما ميكروب الأيروموناس هيدروفيلا أمكن عزله بنســبة ١٦%[عـــترات الأيروموناس هيدروفيلا ٦%، عترات الأيروموناس كافى ٨%، وعـــترات الأيروموناس سوبريا ٢%]. وعند اجراء اختبار حساسية البكتريا المعزولة للمضادات الحيوية المختلفـــة، كانت عترات اليارسينيا أنتيروكوليتكا المعزولة حساسة للمركبات(تتراسيكلين، ستربتومايسين، كلورامفينكـــول والســـلفا ميزوكســـازول) وكـــانت مقاومـــة للمركبـــــات (الأمبيســـــلين والنورفلوكساسين).أماعترات الأيروموناس هيدوفيلا المعزولة فكانت أكثر حساسية للمركبات (كلورامفنيكول، سلفاميزوكسازول، وتتراسيكلين) ولكنها كانت مقاومة للمركبات (الأمبيسلين، النورفلوكساسين والنيومسين).

SUMMARY

In Sohag governorate a trial was made for isolation of *Y.enterocolitica* and *A.hydrophila* from fifty random samples (400 ml each) of pasteurized milk collected from different supermarkets. *Y.enterocolitica* recovered from 4% of pasteurized milk samples. *Y.enterocolitica* isolates were susceptible to Tetracycline (TE_{30}); Streptomycin (S_{10}); Chloramphnicol (C_{30}) and Sulphamethoxazole (SXT_{25}) and resistant to Ampicillin (AM_{10}) and Norfloxacin (NOR_{10}). *A.hydrophila* group isolated from pasteurized milk were *A.caviae* (8%); *A.Sobria* (2%) and *A.hydrophila* (6%). All isolates

were highly susceptible to chloramphnicol (C_{30}); Sulphamethoxazole (SXT_{25}) and Tetracycline (TE_{30}) but resistant to Ampicillin (AM_{10}); Norfloxacin (NoR_{10}) and Neomycin (N_{30}).

Keywords: *Yersinia enterocolitica, Aeromonas hydrophila, pasteurized mik.*

INTRODUCTION

Yersinia enterocolitica and *Aeromonas hydrophila* are currently considered to be of great importance as a foodborn pathogen. Symptoms in human are characterized by diarrhea and abdominal pain in primary stage, but a number of complications or secondary symptoms may also occur (Vidon and Delmas, 1981; Abeyta *et al.*, 1986; Walker, 1987; Stelma, 1988; Jama, 1989; Varnam *and Evans*, 1991 and Palumbo *et al.*, 1992).

The organisms have psychrotrophic nature (able to grow and multiply to large population at normal refrigeration temperature) and they have the ability to produce toxins in the food kept at refrigerator temperature. (Boyce *et al.*, 1979; Stern *et al.*, 1980; Kapperud and Langeland, 1981;Turnbull *et al.*, 1984;Okren *et al.*, 1987; Palumbo *et al.*, 1989; Varnam *and Evans*, 1991 and Freitas *et al.*, 1993; and Kirov *et al.*, 1993. In 1982, Lovett *et al.*, mentioned that, pasteurization process of dairy products recommended by FDA are adequate to destroy large concentration of *Y.enterocolitica.* Moreover they found that the presence of *Y.enterocolitica* in pasteurized milk may probably result from substandard processing or recontamination after pasteurization. Several invetigators could isolated Y.enterocolitica from pasteurized milk (Hughes, 1980; Bimet, 1983; Moustafa *et al.*, 1983; Jamshidian and Babakhani, 1999). Contamination of pasteurized milk even by one organism of *Y.species* could lead to high numbers of such organism during cold storage of milk (Bimet, 1983).

Y.enterocoltica could not survive after pasteurization temperature (62.8 for 30min) of brain heart infustion broth, skim milk and whole milk but could be recovered in few number in peptone sorbital bile broth after 8-10 days of incubation at 10°C (Kushal and Anand, 1999).

Motile *Aeromonas species* associated with human illness are *A.hydrophila*, *A.caviae* and *A.sobria*, (Stelma, 1989). Cousin in 19820, found that several strains of *Aeromonas species* have been isolated from pasteurized milk and the organism increased in

number during cold storage leading to deterioration of milk,while,Tibana *et al.* (1987) stated that, the presence of *Aeromonas species* in white cheese may be due to contaminated pasteurized milk used for production of such product or unsanitary conditions during manufacturing and storage. Several investigators could isolate *A.species* from the pasteurized milk (Drew and Greendway, 1990; Freitas *et al.*, 1993; Kirov *et al.*, 1993; Bahout, 1997 and El-Gamal, 1997) however Melas *et al.* (1999) mentioned that, the *Aeromonas species* could not be detected in the examined pasteurized milk.

. The present study was carried out to investigate the incidence of *Yersinia enterocolitica* and *Aeromonas hydrohydrophila* in pasteurized milk in Sohag city and to study their in vitro sensitivity to different antibacterial agents.

MATERIALS and METHODS

Fifty random pasteurized milk samples (400ml each) were collected from different dairy shops and supermarkets in Sohag city, Egypt. The collected samples were transferred to the laboratory with a minimum of delay to be examined bacteriologically for the presence of *Y.enterocolitica* and *A.hydrophila.*

Isolation:

(I) *Y. enterocolitica:*

Trypticase soy broth tubes were prepared and each tube was inoculated with 1 ml of pasteurized milk samples. The inoculated enrichment broth tubes were incubated at 4°C for 14 days (Greenwood and Hooper, 1989).

Loopfuls from the incubated enrichment broth were streaked directly onto Cefsulodin Irgasan Novobiocin (CIN) agar. The plates were incubated at 35°C for 24h. The suspected organisms were identified morphologically and biochemically according to (Schiemann and Devenish, 1982) (urea hydrolysis, sugar fermentation, kligler iron agar and motility indol lysin sulphide).

(II) *A.hydrophila:*

One ml of each sample of pasteurized milk was added to 10 ml of sterile tryptone soya broth containing 10 μ/ml of Ampicillin in sterile tubes. The inoculated enrichment broth tubes incubated at 28°C for 24h.

A loopful from the incubated enrichment broth was streaked directly onto starch ampicillin agar and cefsulodin irgasan Novobiocin (CIN) agar, then the plates were incubated at 28°C for 24h.

The presumptive aeromonas colonies were subcultured onto nutrient agar slants and incubated at 28°C for 48h. Suspected organism was identified morphologically and biochemically according to Popoff and Veron (1976) (Oxidase test strips, motility test, esculin hydrolysis, gas production from glucose, gelatin liquefaction test and indol production test).

In vitro sensitivity test:

The antibacterial agents used in this study are described in Table 3.

RESULTS

The obtained results are recorded in Tables 1, 2, 3.

Table 1: Incidence of *Yersinia enterocolitica* and *Aeromonas hyolrophila* in the examined pasteurized milk samples.

Number of Samples	*Yersinia enterocolitica* Positive samples		*Aeromonas hyolrophila* positive samples	
	No.	%	No.	%
50	2	4%	8	16%

Table 2: Incidence of *Aeromonas hydrophila* group in examined pasteurized milk samples.

A.hydrophila group	No. of positive samples	%
A.hydrophila	3	6%
A.caviae	4	8%
A.sobria	1	2%
Total	**8**	**16%**

Table 3: In vitro sensitivity testing of *Y.enterocolitica* and *A.hydrophila.*

Antimicrobial discs	*Y.enterocolitica*	*A. hydrophila*
- Streptomycin (S_{10})	S	I
- Amicillin (AM_{10})	R	R
- Sulphamethoxazole (SXT_{25})	S	S
- Chloramphnicol (C_{30})	S	S
- Tetracycline (TE_{30})	S	S
- Cefotaxime (CTX_{30})	I	I
- Norfloxacin (NOR_{10})	R	R
- Neomycin (N_{30})	I	R
- Colistin sulphate (CT_{25})	I	I

S: Susceptible I: Intermediate. R: Resistant.

DISCUSSION

The results recorded in Table 1 revealed that, 2(4%) of the examined pasteurized milk samples were positive for *Y.enterocolitica,* these finding simulate those reported by El-sherbini (1992) while they were lower than those reported by Delams and Vidon (1982) and higher than those recorded by Moustafa *et al.* (1983) Jamshidian and Babakhani (1999).

In vitro, all isolates were susceptible to Tetracycline (TE_{30}); Streptomycin (S_{10}); Chloramphnicol (C_{30}) and Sulphamethoxazole (SXT_{25}) and resistant to Ampicillin (AM_{10}) and Norfloxacin (NOR_{10}).

These results were similar to those recorded by Vidon,and Delmas (1981) Saad and Moustafa (1989) and quite different from those obtained by Adly and Yacoub (1995).

The results recorded in Table 1 revealed that, 8(16%) out of 50 pasteurized milk samples were positive for *Aeromonas species.* These result, were lower than that reported by Freitas *et al.* (1993) and Kirov, et al. (1993) and higher than those recorded by Chen *et al.* (1988); Drew and Greenaway (1990) and El Gamal (1997) but disagree with those recorded by Melas *et al.* (1999) who could not isolate *A.species* from pasteurized milk.

Aeromonas hydrophila; *A.Caviae* and *A. sobria* could be isolated in a percentages of 6%, 8%, and 2% of examined samples respectively. These results were nearly similar to that reported by Bahout *et al.* (1997) [*A.hydrophila* (9.4%), *A.caviae* (11.8%); *A. sobria* (3.5%)], and simulated that reported by Freitas *et al.* (1993) in case of *A.sobria* (2.5%) but lower in case of *A.hydrophila* (12.8%) and *A.caviae* (58.9%).

Aeromonas organisms are sensitive to temperatures above 48°C, there- fore, pasteurized milk are expected to be free of Aeromonas. The presence of such organims in pasteurized milk samples could be attributed to post pasteurization contamination. Palumbo *et al.* (1989). *A.sobria* was more virulant in the cytotoxic activity than the *A.caviae* and *A.hydrophila* (Janda *et al.*, 1984). In vitro, all isolates were susceptible to chloramphnicol (C_{30}); sulphamethoxazole (SXT_{25}) and tetracycline (TE_{30}) and resistant to ampicillin (AM_{10}); Norfloxacin (NOR_{10}) and Neomycin (N_{30}). These results were similar to those recorded by Molero *et al.* (1989)

but were quited different from those obtained by Dixon,and Voran (1992) and El- Gamal (1997).

CONCLUSION

From this study it can be concluded that, some pasteurized milk samples were contaminated by *Y.enterocolitica* and *Aeromonas species* and this may reflect the lack of hygienic supervision and poorly sanitized processing equipment.

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