

THE INCIDENCE OF YERSINIA ENTEROCOLITICA IN DAIRY FOODS

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SUMMARY

Two-hundred random samples of raw milk; pasteurized milk; Damietta cheese and plain yoghurt (50 sample each) were collected from dairy shops and supermarkets in Mansoura city, Egypt, and tested bacteriologically for the presence of *Yersinia enterocolitica* using two different methods for isolation procedure. Of 200 samples tested, 21 (10.5%) were found to contain *Y. enterocolitica* when used BOS without alkali treatment method compared with 26 (13%) samples when used BOS with alkali treatment method. The incidence of *Y. enterocolitica* by both methods from raw milk; pasteurized milk and plain yoghurt was 28% Vs 36% ; 10 Vs 12% and 4% Vs 4% respectively, while *Y. enterocolitica* failed to be isolated from soft cheese using both methods. All *Y. enterocolitica* strains were sensitive to Gentamycin and chloramphenicol, while resistant to penicillin and Ampicillin. All strains varied in susceptibility to Erthromycin, Neomycin, Colistin sulphate and

sulfamethoxazole-Trimethaprim (SXT). The public health importance of isolated organisms as well as the recommended hygienic measures have been discussed.

INTRODUCTION

Food borne diseases have found great interest in recent years. During the late 1970s and the 1980s number of bacteria were generally recognized as food borne pathogens and included *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes* (Walker and Brooks, 1993). *Y. enterocolitica* is a food borne enteropathogenic organism capable of growth at refrigeration temperatures (Walker and Stringer, 1990).

Y. enterocolitica has been isolated from a wide range of foods including raw milk, pasteurized milk, cream, ice cream and cheese (Black et al., 1978; Morse et al., 1984; Tacket et al., 1985 and Schiemann, 1989). The organism is associated with a spectrum of clinical syndromes in man

such as gastroenteritis, terminal ileitis, mesenteric Lymphadenitis, erythema nodosum, arthritis and eye infections (Schiemann, 1989 and walker, 1989). Several reports have indicated that *Y. enterocolitica* could produce toxins in foods (Boyce et al., 1979 and Varnam and Evans, 1991).

The objective of this study is to investigate the incidence of *Y. enterocolitica* in dairy foods and testing their susceptibility to antibiotics.

Materials & Methods.

A total of 200 random samples of raw milk, pasteurized milk, Damietta cheese and plain yoghurt (50 sample each), collected from a number of dairy shops and supermarkets in Mansoura city, Egypt, were examined bacteriologically for the presence of *Yersinia enterocolitica*. All samples were placed in an insulated ice box and examined immediately on arrival at the laboratory.

Isolation of *Yersinia enterocolitica*

Twenty-five ml/g of each sample was added to 225 ml of trypticase soya broth (TSB), then mixed well and incubated at 22 °C for 24h. One ml. of the pre-enrichment culture broth was added to 9 ml. of bile- Oxalate- Sorbose (BOS; Schiemann, 1982), and incubated at 22 °C for a further 5 days. The enrichment culture broth (BOS) was streaked with and without alkali (0.5% KOH solution in water) treatment onto *Yersinia* selective agar (Oxoid CM 653 puls SR 109), incubated at 25 °C and examined 24 and 48 h. (Aulisio et al., 1980)

Typical colonies "bull's-eye" were picked for further identification. Presumptive isolates were screened using kliglers Iron agar (KIA, Oxoid), Urea agar and motility medium incubated at both 37 and 25 °C for 48h. The positive isolates were confirmed using The API 20 E (Biomerieux) and incubated at 30 °C for 24h.

Antibacterial susceptibility testing (Bauer et al., 1966).

All *Y. enterocolitica* strains were performed at 28 °C on nutrient agar by the standard disc method. Antibacterial agents included Ampicillin (10 ug), Penicillin G (10 ug), Carbenicillin (100 ug), Chloramphenicol (30 ug), Erythromycin (15 ug), Gentamycin (10 ug), Nalidixic acid (ug), Colistin sulphate (10 ug); Neomycin (30 ug) and sulfamethoxazole - trimethaprim (SXT, 25 ug).

RESULTS AND DISCUSSION

Incidence of *Y. enterocolitica* reported in Table 1, show that, of 200 samples tested, 21(10.5%) were found to contain *Y. enterocolitica* when plated on *Yersinia* selective medium without alkali treatment Compared with 26 (13%) samples when plated on *Yersinia* selective medium with alkali treatment. Therefore, BOS+ alkali treatment more productive than BOS- alkali method for isolation of *Y. enterocolitica* from raw milk and pasteurized milk (36% Vs. 28% and 12% Vs. 10%) respectively. While there is no difference between the two methods of isolation in case of plain yoghurt (4% on both methods). *Y. enterocolitica* failed to be detected in Damietta

cheese samples using both methods.

These findings are in agreement with those reported by Umoh et al. (1984), Walker and Gilmour (1986), Hafez, (1988), Moustafa, (1990) and Walker and Brooks, (1993). High incidence percentages were reported by Vidon and Delmas (1981) who found that 81.4% of raw milk samples contained *Y. enterocolitica* while Moustafa et al. (1983) reported that of 100 raw milk samples tested, 12 samples contained *Y. enterocolitica*.

Y. enterocolitica gained entry into milk via contaminated equipment, dust or any other pathway it could then multiply during refrigerated storage prior to pasteurization. Considering the large volumes of milk and cream passing through a commercial pasteurization plant it may be possible for a few organisms to survive pasteurization and to multiply in the refrigerated pasteurized product, especially if pasteurization temperatures are not strictly observed. Alternatively *Y. enterocolitica* may gain access to the pasteurized milk by post-pasteurization contamination from raw milk (Greenwood et al. 1975). While the possible sources of the organism in yoghurt are contamination of the mother culture, contamination by handlers or post pasteurization contamination (Umoh et al. 1984).

Data on the recovery rate of *Y. enterocolitica* from raw and pasteurized milk with each two methods (Table, 2) revealed that post enrichment

treatment with alkali was effective for recovery of *Y. enterocolitica* isolated from raw milk (23 Vs. 17 isolates) and pasteurized milk (8 Vs. 6 isolates), while the isolation rate of *Y. enterocolitica* was similar for both methods in plain yoghurt samples (2 isolates each).

Y. enterocolitica prevalence in raw milk and ability to survive and multiply at low temperatures make milk a possible vehicle for yersiniosis. However, the organism is considered sensitive to low pH. Aytac and Ozbas (1994) monitored the survival of *Y. enterocolitica* during production and storage of yoghurt. The *Yersinia*, however, grew during the first few days and survived throughout the observation period even though the pH of the yoghurt fell rapidly to 4.3-4.4. So, I think that probably the post enrichment with alkali treatment not play important role for recovery of yersinias from yoghurt samples due to low pH of yoghurt.

All 58 isolates were resistant to Penicillin and Ampicillin but they were sensitive to chloramphenicol and Gentamycin. More than half were sensitive to Erythromycin (79.3%); Neomycin (71%); Colistin sulphate (65.5) sulfamethoxazole - Trimethaprim 57% and Nalidixic acid (51.7). While only 10 strains were sensitive to Carbenicillin (Table 3).

Nearly similar results were obtained by Frazin et al., 1984; Umoh, 1984; Nagah and Sabah, (1989 and Alzugaray et al., 1995).

In conclusion; It is clear that transmission of

Table (1): Incidence of *Yersinia enterocolitica* in examined samples

Dairy foods	No. of Samples	Incidence % BOS without alkali treatment		Incidence % BOS without alkali treatment	
		+ve samples	%	-ve samples	%
Raw milk	50	14	28	18	36
Pasteurized milk	50	5	10	6	12
Damietta cheese	50	--	--	--	--
Plain yoghurt	50	2	4	2	4
Total	200	21	10.5	26	13

Table (2): frequency distribution of *Yersinia enterocolitica* isolates

Dairy foods	Frequency % BOS without alkali treatment		Frequency % BOS without alkali treatment	
	No. of isolates	%	No. of isolates	%
Raw milk	17	68	23	69.7
Pasteurized milk	6	24	8	34.3
Damietta cheese	--	--	--	--
Plain yoghurt	2	8	2	6
Total	25	100	33	100

Table (3): Antibacterial sensitivity for *Y. enterocolitica* isolates (58 strain) obtained from examined dairy foods

Antibiotic drugs (ug)	No. of sensitive isolates	%
Penicillin G (10)	0	00
Ampicillin (10)	0	00
Carbencillin (100)	10	17.20
Chloramphenicol (30)	58	100.0
Erythromycin (15)	46	79.30
Gentamycin (10)	58	100.0
Nalidixic acid (30)	30	51.7
Colistin sulphate (10)	38	65.5
Neomycin (30)	41	71.0
Sulfamethoxazole	33	57.0
Trimethoprim (SXT, 25)		

Yersiniosis from contaminated raw milk and its products is expected. The post-enrichment alkali treatment used in this study appear to be a reliable procedure for recovery of *Y. enterocolitica*. Therefore, strict hygienic measures should be adopted during production, handling and processing to prevent contamination of raw milk as well as proper heat treatment and the use of suitable starter cultures would eliminate the risk of infection from this organism and to ensure a maximum of safety to the consumer.

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