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EVALUATION OF RAW MILK FOR ANTIBIOTIC RESIDUES

(With 2 Tables)

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تقييم اللبن الطازج لوجود بقايا المضادات الحيوية

عزه على حسين التابعى

في دراسة لتقييم اللبن الطازج من حيث وجود بقايا المضادات الحيوية، تم جمع ٧٥ عينة عشوائية من اللبن الطازج بأسواق مدينة بورسعيد، وقد تم فحص هذه العينات لبقايا المضادات الحيوية وذلك باستخدام طريقة اختبار الدنفو. وقد وجد أن ٨ عينة (١٠,٧%) من إجمالي عدد العينات كان إيجابياً لوجود المضادات الحيوية منهم ٣ عينة (٤%) إيجابية للبنسلين. وبدراسة تأثير الغليان على المضادات الحيوية في اللبن الطازج وجد أن عدد ٢ عينة (٢٥%) من إجمالي عدد العينات الإيجابية التي أعيد اختبارها كان إيجابياً، مما يعني أن الغليان له تأثير فعال على تقليل نسبة وجود المضادات الحيوية في اللبن الطازج. وقد تم مناقشة تأثير بقايا المضادات الحيوية على الصحة العامة.

SUMMARY

Seventy five samples of raw milk, collected from different local markets in Port Said city, were examined by Delvo test-P for detection of antibiotic residues. Eight (10.7%) of raw milk samples were found to contain antibiotic residues. Penicillin was found in 3 (4%) of positive samples. After boiling for 10 minutes, only 2 (25%) samples were positive for antibiotic residues. The occurrence of antibiotic residues in milk were discussed with regard to public health.

Key words: Milk, antibiotic residues

INTRODUCTION

Milk besides it is a finely dispersed and easily assumable, is considered as the most really perfect single foodstuff for both infants and adults.

Antibiotic residues paid greatly the attention of the food hygienists all over the world due to the wide uses of antibiotics in animal breeding as a prophylactic effect against many farm diseases and/or as therapeutic uses against some diseases specially those closely associated with cessation in milk production, it also maintain and promote growth (Allison 1985). Antibiotic residues may also be found as natural constituents such as antibiotics synthesized by the lactic acid bacteria (Friend and Shahani 1983). Presence of antibiotic residues in milk may be due to failure with holding milk for the full withdrawal period (Booth and Harding 1986).

Use of unauthorized antibiotics or the failure to follow label direction for approved antibiotics could results in unsafe-antibiotic residues in food products, will potential adverse effects on human health (Pena *et al.*, 1999). The passage of antibiotics into milk from medicated animals causes major problems of the quality of raw milk. Some antibiotics can cause idiosyncrotic reaction in ultrasensitive consumers and their exposure may lead to an increase in the numbers of resistant to antibiotic individuals (Dewdney *et al.*, 1991). In addition antibiotics can delay (if not totally prevent) the bacteriological processes used in the manufacture of certain dairy products and influence negatively coagulation processes (Grunwald and Petz 2004), and affecting the flavour and texture of such products (Ripley 1999).

Over the last several years, there have been difficulties for rapid detection of antibiotic residues in milk, beside farmers asking about withdrawal and residues prevention methods (McEwen *et al.*, 1991). Therefore the development of analytical methods of low levels of antibiotic residues in milk is necessary. Recently modified delvo test-P method has been evaluated in several investigations with accurate satisfactory results (Carlsson and Bjorck, 1991). The delvo test -P method is qualitative because there is only change in its colour indicator when acid is produced by the microorganism via metabolic processes under conditions of uninhibited growth.

The present study was performed to investigate the presence of antibiotic residues in raw milk in Port-Said city as well as to study the effect of heat treatment (boiling) on such residues.

MATERIALS and METHODS

1. Samples collection:

The milk analyzed in this survey was obtained from different markets in Port Said city, all samples were received in sterile capped

bottles and immediately placed into an insulated icebox, the temperature being kept at 4°C, and transferred to the laboratory with a minimum of delay for detection of antibiotic residues in raw milk samples.

The samples were tested for detection of heat-treated milk using Storch's test to prove that milk samples were raw, (Lampart, 1975).

2. Preparation of samples:

Each milk sample was heated at 82°C for 5 minutes in water bath to inactivate the natural inhibitors in milk to avoid false positive results after heating at 82°C for 5 min, milk samples were rapidly cooled to 20°C with cold water (Oliver *et al.*, 1990 and Kang *et al.*, 2005).

Each milk sample was examined for the presence of antibiotic residues using Delvo test-P method (Tube Diffusion Method with *Bacillus stearothermophilus* var. *calidolactis* as test organism), which is highly sensitive to most antibiotics.

3. Testig procedure:

The technique applied was recommended by A.P.H.A, (1985). The Delvo test-P kit was obtained from Gist brocades, Delft, Holand. The method applied followed instruction supplied with the test kit materials. One nutrient tablet was add to each ampule using the tweezers and 0.1 ml of milk sample was introduced through the neck of the ampule using a new disposable syringe tip for each ampule. A positive control (0.1 ml of penicillin standard solution 0.005 I.U./ml), and negative control (free inhibitor skimmed milk) were made. The test ampules were incubated in water bath adjusted at 64°C for 2-3 hours until the negative control showed a yellow colour. Penicillinase enzyme was added to the samples which showed positive results for antibiotic residues in order to detect the presence of penicillin.

3.3 Effect of heat treatment on positive samples:

Positive samples of antibiotic residues were boiled at 100°C for 10 minutes and reexamined for the presence of antibiotic residues.

RESULTS

Table 1: Incidence of antibiotic residues in raw market milk samples as detected by Delvo test-P.

No. of examined samples	Positive samples		Negative samples		Samples positive for Penicillin	
	No.	%	No.	%	No.	%
75	8	10.7	67	89.3	3	4

Table 2: Incidence of antibiotic residues in boiled market milk samples as detected by Delvo test-P.

No. of examined samples	Positive samples		Negative samples		Samples positive for Penicillin	
	No.	%	No.	%	No.	%
8	2	25	6	75	0	0

DISCUSSION

Antibiotic residues can appear in milk from several routes: mastitis treatments, injectables, feeds contaminated with antibiotics, inappropriate feeding of antibiotics, bolluses used post calving to prevent infections, and addition of drugs to drinking water (Brady and Katz, 1988).

Heat treating milk prior to testing may reduce the number of false positive reactions for some test methods (Kosikowski, 1963). Cogan and Fitzgerald (1980) advised heating milk samples to 82°C for five minutes to destroy any natural inhibitors occurring in milk. The presence of natural inhibitors lysozyme in milk is correlated with the presence of large numbers of neutrophilic granulocytes, as for instance in colostrum and mastitic milk, the presence of such inhibitors may be one of the factors responsible for the occasional false positive result encountered in the assay for antibiotic residues (Egan and Meaney 1984).

The summarized results in Table (1) showed that the incidence of antibiotic residues in raw milk samples was 8 (10.7%) out of 75 examined samples. Three of them were positive for penicillin (4%). Variable findings were reported by Tseng, (1974), Jurdi and Asmar (1981), Hafez *et al.* (1990), Calhau *et al.* (1992) and El-Kohly *et al.* (1994).

One of the hazards of penicillin residues: the development of acquired resistance is reported in some bacteria due to production of penicillinase enzyme (Huber *et al.*, 1969 and Katz *et al.*, 1974).

The presence of antibiotic residues in raw milk may be due to extra-label use or misuse of antibiotics in animals by farmers and also results of insufficient withdrawal time after drug administration. In this concern, A.P.H.A. (1985) reported that milk of the treated animals is not supposed to be used for human consumption for at least 72 h. following last treatment. The WHO/FAO guidelines for antibiotic residues in milk for human consumption limit most residues to <0.2 ppm I.D.F. (1991).

Regarding to the effect of boiling on detection of antibiotic residues, data, recorded in Table (2) revealed that 2 (25%) samples out of 8 (10.7%) of raw milk which were boiled to 100°C for 10 minutes gave positive results. Results showed that the positive samples for antibiotic residues decreased in number after heat treatment, probably due to the denaturalization effect of heating on certain indigenous antibacterial substances present in milk. Koncey (1978) and Tropilo (1985) stated that the absence of penicilline in heat treated milk may be due to the inactivation of penicillin by heat.

There are regulations of veterinary drugs used in most countries. The Commission of European Communities (CEC) regulation 675/92 stated the maximum residue limits (MRLs) for a number of antibiotic based on their toxicological and technological significance (Heeschen and Suhren 1993). In some developing countries, there is no national program for routine monitoring of residues in food animals.

The presence of antibiotic residues in milk cause several problems include allergic reactions and even toxicity as well as development of resistant strains of bacteria (Booth and McDonald, 1988 and WHO, 1988). High levels of residues constitute a potential and real public health risk for consumers. One of the harmful effects is direct toxicity, such as liver toxicity seen with chloramphenicol, and neurotoxicity associated with streptomycin (Pennycott 1987). On the other hand, the prolonged exposure to some of these antibiotic residues leads to possible pharmacological toxicological (teratogenicity, carcinogenicity and mutagenicity) microbiological (pathogenic microorganisms) and immunopathological (allergic) effects (Heeschen and Suhren, 1993, Jensen, 1995, Hubbert *et al.*, 1996 and proto, 1997). Therefore it is importance to avoid the presence of these residues in milk in order to reduce problems during processing as well as to prevent their transmission to the consumers.

The present study has highlighted this potential hazard by the presence of detectable antimicrobial residues in milk. So veterinarians were able to advice farmers on the problems of residues in milk and inform them about withholding times for milk collected from treated cases depending on drug label instructions as well as periodical analysis of milk samples. Therefore it is important for health authorities in our country to impose detection and regulation of antibiotics at receiving stations or dairy plants.

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