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EVALUATION OF MICROBIAL QUALITY OF SUPPLIED IN LORESTAN PROVINCE, WEST OF IRAN

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ABSTRACT: Outbreaks of food borne diseases has always been one of the world problems. Raw and pasteurized milk contamination can occur because of various reasons, even in developed countries. Microbial total count in milk evaluating has not only been important in its suitability for human consumption but more important in causing illness in its consumers. The aim of this study was to evaluation of microbial contamination of the milk supplied in Lorestan province. This study was implemented during March to April 0f 2013 on 97 collected samples. Total bacteria, coliforms and Escherichia coli were enumerated with specialized standard microbial tests. Obtained results of this study showed that 78% of samples were acceptable according to Iran standard and consumable witch is a good indicator of microbial quality of milk in Lorestan province. Our study showed that the supplied milk was in a good hygienic condition. To increase the milk quality the following actions are necessary: Training, supervision on milk obtaining, good transferring, hygienic production and maintenance, continuous monitoring by health centers and government agencies.

Key words: Milk, Microbial load, Supply level, Lorestan Province, Iran

INTRODUCTION:

Outbreaks caused by the food consumption have always been a problem throughout the world. Contamination of foods with Foodborne pathogens arise foodborne diseases. Industrialization improved health, housing, nutrition and not only reduced the number of patients and mortality rate but also has increased population life expectancy (WHO, 2002; Daniels *et al.*, 2002).

Nowadays, foodborne diseases are one of the most important problems of human societies especially in developing countries and held back countries. Infectious diseases are responsible for 45 percent of deaths in poor countries, and half of the early deaths throughout the world (CDC, 1990; Todd, 1996; Loir *et al.*, 2003).

Estimating of prevalence of food-borne diseases is difficult, it was reported that 2.1 million people died from diarrheal diseases in 2000. A large proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2006).

Common symptoms of food-borne diseases are diarrhea, fever, headache, vomiting, abdominal cramps, fatigue, and sometimes blood and pus in the stool (FSIS, 2000). According to Centers for Disease Control, foodborne diseases causing serious illness leading to hospitalization, gastrointestinal diseases and 500 million deaths per year (Mead *et al.*, 1999).

Raw and pasteurized milk contamination problem can occur for various reasons, even in advanced societies. Total microbial count in milk evaluating has not only been important in its suitability for human consumption but more important in causing illness in its consumers (ISIRI, 2014).

Milk is one of the most important items of family's diet, therefore bacteriological control of raw and pasteurized milk is worth and very important in the centers of production, collection and processing of milk (Sadeghifard *et al.*, 2006).

Coliforms are gram-negative, non-spore-forming, rod-shaped, aerobic or anaerobic bacteria which are capable of fermenting lactose to producing acid and gas at 30 to 37 ° C during 24 to 48 hours (Barot, 1983).

Some strains of Escherichia coli as a Gram-negative bacterium of the Entrobacteriacea family cause gastroenteritis, septicemia, urinary tract infection and meningitis. Escherichia coli are found in the intestinal contents of humans and animals, presence of them in the outside of the intestine indicated contamination with human or animal feces. Isolation of E. coli from foods shows foods contamination with fecal microorganisms (Akhavan sepahi, 2006).

Raw milk hygienic quality can play a significant role in the quantity and especially the quality of the obtained milk products (Ma and Barbano, 2003; Ma and Barbano, 2003).

Milk and dairy products are the most important sections of the diet, the aim of this study was carried out to evaluate the microbial quality of supplied milk in Lorestan province to improve public health standard.

MATERIALS AND METHODS:

Sampling

This study was implemented during March to April of 2013. 97 sample of supplied milk provided randomly. Samples were sent to the laboratory, immediately. and various tests including total bacteria count, coliform and E. coli bacteria count tests were carried out according to the Iran national standards protocol.

Samples with more contamination than standard limits were declared non-consumable and samples with low levels were declared acceptable. Standard limits are listed in Table 1.

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Table 1.

Limits and standards for milk contamination

Contamination limit	Bacteria
<1000	Total count
<10	Coliforms
Negative	E.coli

Microbial tests

Identification of Escherichia coli in pasteurized milk (Iran national standard No. 2946)

1 mL of liquid sample was added to tubes contained 10 ml LST Broth with Durham tubes. Tube was incubated at 37 ° C for 24 to 48 hours. If gas or turbidity was seen in this tube, 1- 2 drops were added to other tube containing 1 to 10 mL of EC Broth with Durham tubes, and incubated at a temperature of 44 to 45 ° C for 24 to 48 hours (ISO, 2005). If gas or turbidity was seen in the last tube, 1-2 drops of it added to a tube contained peptone water without indole and was incubated at 44 to 45 ° C for 24 to 48 hours in a bain marie. 0.5 mL of Kovac's reagent added to the tube and appearance of red color in the culture medium (positive indole reaction) to be determined.

The EC broth medium containing was streaked on to mac conkey agar medium and purple lactose-positive colonies were analyzed. Streaking of the colonies on nutrient agar medium to create isolated colonies was examined for confirmatory testing. Confirmatory tests include culture and differentiation of TSI, indole, methyl red, citrate Simon (IMVIC) tests (ISO, 2005).

Total count of microorganisms in pasteurized milk at 30 ° C (Iran National Standard No. 5272)

1.0 mL of required serial dilution was transferred to empty plates and 15 to 20 ml of plate count skim milk Agar (pcsA medium) was added and pour plated a layer at 45 to 50 °C and were mixed gently. In the possibility of microbial growth on the surface of the medium with the expanded colonies, the medium is covered with a thin layer of growing medium and made of two layers. Agar plates upside down and was incubated at 30 ° C for 72 hours. The growth of microorganisms such as bacteria, mold and yeast was counted on each plate with using the following formula (ISO, 2003).

Colonies number (mL) = total number of colonies × dilution-1 × volume-1 (ISO, 2003).

The average number derived from the total count of microorganisms was report at 30 ° C. In milk and milk products samples colony count should be done in soft light and not to be confused with the deposited particles because of addition of nonfat dry milk (0.01) to culture medium (16). Standard limit to aerobic microorganisms is 7.5 × 104 cfu/ml (ISO, 2003).

Enumeration of coliforms in pasteurized milk at 30 ° C (Iran National Standard No. 5486-1)

1.0 ml of the diluted milk samples were added to sterile empty plate. 15 ml of Violet Red Bile Lactose (VRBL) Agar that is cooled to a temperature of 45 ± 1 ° C was poured on the plates and was mixed carefully. Plates were placed on a flat surface. After complete solidification of the medium, about 4 ml of culture medium were added to the previous layer. Plates upside down and were incubated at 30 ° C for 24 ± 2 hours. Reddish purple colonies with a red halo (caused by deposition of bile) were counted.

Non-specific colonies were transferred into tubes containing 10 ml of Brilliant Green Bile Broth medium and Durham tubes for confirmation. Then tubes were incubated at 30 ° C for 24 ± 2 hours. If gas was formed in the tubes, Non-specific colonies were counted in the first plates. If turbidity with no gas formation was seen in tubes, a certain amount of tubes was streaked on mac conkey agar and reddish purple colonies were counted after incubation. Coliforms count was reported by the use of following formula (ISO, 2004):

Coliforms number (mL) = total number of colonies (confirmed and non-specific colonies) × dilution-1 × volume-1

The number of colonies in a milliliter sample specifiable number of colonies were counted and colony confirmed shots × dilution × image size is used (ISO, 2004). Allowable level of coliforms in pasteurized milk ml 10cfu / is (ISO, 2004).

RESULTS:

The obtained results showed that 78% of samples were acceptable and consumable according to the Iran's national standard. 90% of the samples were negative for E. coli. Results and details of experiments are showed in Table 2.

Contamination Level of the collected milk samples from the Lorestan province have been reported in Table 3.

Table 2.

Results and details of microbial analysis of milk samples

Number	Total count	Coli form	Escherishia coli	Acceptable/Unacceptable
1	7×10^4	uncountable	Positive (+)	Unacceptable
2	3.2×10^3	<10	negative	Acceptable
3	3.7×10^3	<10	negative	Acceptable
4	10^2	<10	negative	Acceptable
5	2×10^3	<10	negative	Acceptable
6	2×10^3	3.2×10^3	negative	Unacceptable
7	8×10^2	<10	negative	Acceptable
8	1.1×10^3	<10	negative	Acceptable
9	8×10^2	<10	negative	Acceptable
10	$<10^3$	<10	negative	Acceptable
11	3×10^2	<10	negative	Acceptable
12	5×10^2	<10	negative	Acceptable
13	4×10^4	4×10^4	negative	Unacceptable
14	10^3	<10	negative	Acceptable
15	7.3×10^3	<10	negative	Acceptable
16	3×10^2	7×10	negative	Unacceptable
17	7×10^4	uncountable	Positive (+)	Unacceptable
18	6.4×10^4	uncountable	Positive (+)	Unacceptable
19	7×10^4	uncountable	Positive (+)	Unacceptable
20	5.5×10^4	uncountable	Positive (+)	Unacceptable
21	6.2×10^4	uncountable	Positive (+)	Unacceptable
22	4×10^3	<10	negative	Acceptable
23	3.5×10^3	<10	negative	Acceptable
24	10^4	4.1×10^2	negative	Acceptable
25	8×10^3	3.5×10^2	negative	Acceptable
26	1.2×10^3	<10	negative	Acceptable
27	2×10^3	<10	negative	Acceptable
28	10^3	<10	negative	Acceptable
29	1.2×10^3	<10	negative	Acceptable
30	1.2×10^3	<10	negative	Acceptable
31	8×10^2	<10	negative	Acceptable
32	2×10^3	2.5×10^2	negative	Unacceptable
33	1.6×10^3	6×10	negative	Unacceptable
34	1.5×10^3	<10	negative	Acceptable
35	8×10^2	<10	negative	Acceptable
36	10^2	<10	negative	Acceptable
37	2×10^2	<10	negative	Acceptable
38	1.5×10^3	<10	negative	Acceptable
39	8.5×10^3	1.2×10^2	negative	Unacceptable
40	7×10^3	1.5×10^3	negative	Unacceptable
41	3×10^3	<10	negative	Acceptable
42	3×10^3	<10	negative	Acceptable
43	7×10^2	<10	negative	Acceptable
44	10^3	<10	negative	Acceptable
45	8×10^2	<10	negative	Acceptable
46	2.5×10^3	<10	negative	Acceptable
47	6×10^2	<10	negative	Acceptable
48	3×10^2	<10	negative	Acceptable
49	8×10^2	<10	negative	Acceptable
50	10^3	<10	negative	Acceptable
51	8×10^3	<10	negative	Acceptable
52	6×10^3	<10	negative	Acceptable
53	1.3×10^3	<10	negative	Acceptable
54	8.2×10^3	6×10	Positive (+)	Unacceptable
55	6.4×10^3	7×10	Positive (+)	Unacceptable
56	6.7×10^3	3×10	Positive (+)	Unacceptable
57	5.3×10^3	5×10	Positive (+)	Unacceptable
58	1×10^3	<10	negative	Acceptable
59	2×10^2	<10	negative	Acceptable
60	7.2×10^3	<10	negative	Acceptable
61	8×10^3	<10	negative	Acceptable
62	2.1×10^3	<10	negative	Acceptable

63	10^3	<10	negative	Acceptable
64	7×10^2	<10	negative	Acceptable
65	3×10^2	<10	negative	Acceptable
66	10^3	<10	negative	Acceptable
67	8×10^2	<10	negative	Acceptable
68	3×10^2	<10	negative	Acceptable
69	3×10^2	<10	negative	Acceptable
70	5×10^2	<10	negative	Acceptable
71	10^3	<10	negative	Acceptable
72	2.8×10^3	<10	negative	Acceptable
73	2.3×10^3	<10	negative	Acceptable
74	8×10^3	<10	negative	Acceptable
75	5×10^2	<10	negative	Acceptable
76	3×10^2	<10	negative	Acceptable
77	2.7×10^3	<10	negative	Acceptable
78	10^4	<10	negative	Acceptable
79	1.1×10^4	<10	negative	Acceptable
80	6×10^3	10^3	negative	Unacceptable
81	6.2×10^3	1.5×10^2	negative	Unacceptable
82	8×10^2	<10	negative	Acceptable
83	1.4×10^2	<10	negative	Acceptable
84	7.5×10^3	<10	negative	Acceptable
85	10^3	<10	negative	Acceptable
86	8×10^2	<10	negative	Acceptable
87	10^3	<10	negative	Acceptable
88	1.5×10^2	<10	negative	Acceptable
89	1.4×10^2	<10	negative	Acceptable
90	1.2×10^2	<10	negative	Acceptable
91	4×10^3	4×10	negative	Unacceptable
92	4×10^3	6×10	negative	Unacceptable
93	8×10^3	<10	negative	Acceptable
94	1.2×10^3	<10	negative	Acceptable
95	9×10^2	<10	negative	Acceptable
96	9×10^2	<10	negative	Acceptable
97	1.2×10^2	<10	negative	Acceptable

Table 3.
Contamination level in collected milk samples of Lorestan provinces in Iran

Contamination with	Percent (%)
Total count	22%
Coliforms	6%
E. coli	10%

DISCUSSION:

Food borne diseases outbreaks have always been a problem throughout the world and improving the disease are very expensive every year. Milk and milk products have great potential to microbial contamination due to their ingredients and conditions for extraction, transmission and their maintenance conditions.

The obtained results indicate that contamination of milk with enteric gram-negative bacilli of the Enterobacteriaceae family is low (22%). This contamination level can be due to person's hygiene who has worked milk production centers. Milk and milk products handlers capable of transmitting microbes through contact with raw material (milk).

Many studies were done on microbial contamination of milk and its products.

Arab Ameri and coworkers in shahrood city of Iran showed 61% of raw milk and 5% of pasteurized milk contamination with E. coli (Arab amery, 2007). In another study in Yazd city of Iran,results showed that 81.3% of milk samples were acceptable and consumable which is corresponded with our obtained results (Salari *et al*., 2007).

In a study by Desai and colleagues in India, high microbial load of milk was reported from 124 milk collection centers (Desai and Natarajan, 1981). Obtained results in this study have a significant difference with the findings of present study. In other study in Bulgaria for the evaluation of coliforms and pH of raw milk, results showed that 0.2% of samples had high acidity and average microbial load of samples was 107 (Aleksieva and Krushev, 1981).

Kenya is one of the area that most dairy farmers produce high quality milk, but because of the distance, high temperature environment, improper washing the dishes, microbial load of collected milks increased (Ombui, 1995).

Vahmni and colleagues in evaluating the microbial quality of raw milk in 2 forms bulk and during delivering to dairy factories in the kerman province of

Iran, stated that microbial loads was 37.31 × 106 and 7.47 × 106 cfu/ml, respectively (Vahmni, 2003).

In a study in Ethiopia on the microbial quality of raw milk, total count of milk during collection and storing, during cool down and dairy factories delivering was 1.1× 105, 4 × 10 and 1.9 × 108 cfu / ml, respectively (Godefay and Molla, 2000).

Results of a study in Brazil showed that the microbial load of raw and pasteurized milk and coliform contamination in 60 samples of milk was due to improper washing of storage tank and improper temperature (Lopes and Stamford, 1997). Evaluation of the microbiological composition of raw milk from Normandy region of France showed coliforms were observed in the majority of samples and contamination of 84% of them were with coliforms and E. coli less than 102 and less than 10 cfu/ml, respectively (Desmasures *et al.*, 1997). Results of a Survey on South Dakota dairy farms in America showed contamination of 62.3% of samples with coliforms (Jayarao and Wang, 1999).

Food borne diseases indicate prevalence and extension of public health problems in developed and developing countries. However, this problems have more effects on the health and economic in developing countries than in developed countries (Notermans *et al.*, 1995). Studies have shown that educating and raising people's awareness has very effective role in improving food hygiene (Zare and Shojaie-zadeh, 2001).

Studies which have been conducted in recent years showed that adding CO2 to milk is an effective way for preventing the increasing microbial load and control of Psychrotrophic microbes in raw milk (Hotchkiss *et al.*, 1999). Combination of CO2 and low temperature improves raw milks hygiene and microbial quality (Rajagopal *et al.*, 2005). Health measures and education about letdown stage and various stages of raw milk collection are very effective in the reduction of contamination and elimination of foci of pollution (Teymori *et al.*, 2014; Asadzadeh *et al.*, 2014; Haghighat-Afshar *et al.*, 2014; Teymori *et al.*, 2014).

Quality is an essential component in dairy industry which is worth more than production quantity. Milk quality is effective on all stages of production, collection and storage of the product. Non-compliance of quality requirements will reduce production and products revenue.

CONCLUSION:

Based on the obtained results, it can be concluded that the contamination of milk should be discussed as a new topic. Further investigations need to reach the standard limits. Continuous monitoring and suitable controlling will reduce food contamination to zero in order to ensure public health.

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