Microbial quality assessment of pasteurized milk of supplied to Lorestan province market, Southwest of Iran

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ABSTRACT

Objective: Bacterial contamination and food poisoning is one of contemporary issues in the early years which many governments like Iran allocate some million dollars a year to medical and social treatment to prevent the implications.

Methods: This paper aims to determine the bacterial load in pasteurized milk, produced for Lorestan province market in 2014. During the course of the year, 118 samples were collected and sent to the official laboratory.

Results: According to the Iran standard testing protocols, 11% of samples exceeded acceptable range.

Conclusions: The diversity of reports is because of different range of personal hygiene, delivery system quality, maintaining equipment and regards for milking. Beside these factors, season of sampling may affect the subject.

KEY WORDS: Pasteurized milk, Lorestan province, Iran, Escherichia coli, Coliforms

1. INTRODUCTION

Food and nutrition are essential to live but it should be noted that, this crucial role is eclipse when foodborne pathogens get involve. Then, they may cause some disorders instead of promoting growth (Shams, 2002). All of nutrients are exposed to physical, chemical and biological contaminations (Giti, 1991). Milk as a rich nutritional environment encourage proliferation of microorganism (Laval, 1995). Milk contamination impact both nutritional content and health of consumers (Nanu, 2007). Different unprotected condition threatens hygiene standard of milk which the post pasteurization factors have attracted more often attention (Rahman, 2015). However, quality of row milk and sanitary condition of milk products’ industry are the most important treat which influence sanitary quality of dairy products (Herrera, 2014).

Results of studies shown which foodstuff infectious and human food born diseases as the most well-known diseases have become more common and cause suffering human with serious problems, especially in undeveloped countries (Fatholahzadeh, 2009; Asadollahi, 2011; 2012; Taherikalani, 2011; 2008; Emaneini, 2009; Jabalameli, 2011; 2012; Sorosh, 2010; Pakzad, 2011; Shahsavani, 2012; Haghi-Ashtiani, 2007; Khoramrooz, 2012; Akbari, 2010; Sahebekhtiari, 2011; Kalantari, 2007; Nakhjavani, 2013).

Many researches have demonstrated that about 70% of infectious diseases are related to unhygienic foods. More than 450 bacteria, viral, fungal and parasitical infections have transmitted to human through animal source foods. (Marandi, 1999). The presents of some indicator microorganisms are the criteria for verifying appropriate manufacturing process and hygienic production condition (Flores, 1999). Total coliform bacteria investigation is one of common test for evaluation fecal contamination (Vahedi, 2015).

Coliforms are known as normal flora of intestinal track. Determination of coliforms in milk indicates negligence in carry out sanitary procedure in distribution, transportation and preparation systems (ISO, 2002). Salmonella spp., Staphylococcus aureus, and E. coli are the most common pathogens involving in milk contamination (Angulo, 2009; Vahedi, 2015). This study aims to assess sanitary condition of milk producer factories and to prove the necessity of improvement in systems of control and protection.

2. MATERIALS AND METHODS

2.1. Sample collection: During March to April 2014, 118 pasteurized milk samples were collected at random from different producer in Lorestan province. The samples were transmitted to laboratory and were tested for load of total bacteria, coliform and E. coli according to the Iran national standard protocol. By considering standard limits (table.1) two types of samples were defined acceptable and inconsumable which respectively referred to samples with lower and higher level of contamination. The samples were prepared by sterilized equipment and were preserved in 4 degree Celsius before running the tests.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Acceptable limit (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count</td>
<td>&lt;1×10³</td>
</tr>
<tr>
<td>Coliforms</td>
<td>&lt;1×10¹</td>
</tr>
<tr>
<td>Aerobic microorganisms</td>
<td>&lt;5.7×10⁴</td>
</tr>
<tr>
<td>E-Coli</td>
<td>0 (Negative)</td>
</tr>
</tbody>
</table>

2.2. Microbial tests: All culture media and materials were purchased from Merck Company.
2.3. Identification of Escherichia coli in pasteurized milk (Iran national standard No. 2946): 1 ml milk sample was diluted ten times in a tube which contained 10 ml LST Broth and was equipped with Durham tube for gas production determination. The tube was incubated for 24 to 48 hours at 37°C temperature. If gas production or turbidity were observed, 1-2 drops of liquid culture were added to other tube which contained 10 ml EC broth and Durham tube and were incubated at 44 to 45°C for 24 to 48 hours (ISO, 2005). If gas production or turbidity were observed again, 1-2 drops of suspension culture were added to tube contained peptone water missed indole and were incubated at 44 to 45°C for 24 to 48 hours. Then, 0.5 ml Kovac’s indicator were added the tube. The positive reaction caused red color appearance otherwise the culture medium didn’t change. Mac conkey agar medium was used for streak culture of EC broth suspension and lactose-positive colonies with purple color were isolated and cultured on nutrient agar medium. E.coli species recognition were confirmed by running TSI, indole, methyl red, simon citrate (IMVIC) tests.

2.4. Total count of microorganisms in pasteurized milk at 30°C (Iran National Standard No. 5272): In order to counting total number of aerobic and anaerobic microorganisms in sample, 1.0 ml of diluted series was poured into empty plates. Then 15 to 20 ml of pcsmA medium (plate count skim milk Agar) at 45 to 50 °C were added and shaken carefully. The medium of surface was covered with an extra thin layer in case of aerobic colonies expansion on growth medium. The plates were inverted and incubated at 30°C for 72 hours. Total number microbial contaminations were calculated by equation 1 (ISO, 2003) and the average amount was reported.

\[ \text{Eq.1: Colonies number (cfu/mL)} = \text{total number of colonies} \times \text{dilution-1} \times \text{volume-1} \]

Colony counting was carried out under adequate light condition to avoid being mistaken about milk powder sediments particles of medium and colonies.

2.5. Enumeration of coliforms in pasteurized milk at 30°C (Iran National Standard No. 5486-1): As it was described, a dilution series of milk samples were prepared and 1.0 ml of them were poured into empty plates. Then, 15 ml of VRBL (Violet Red Bile Lactose) at 45 ± 1 °C agar were added and mixed softly. When the medium cooled down and became solid about 4 ml of culture medium were added to cover the medium surface.

The inverted plates were incubated for 24 ± 2 hours at 30°C. The purple colonies with central red spot caused by bile particle accumulation were easily recognized as coliform and were counted. The uncertain colonies were confirmed by gas production in Brilliant Green Bile Broth medium at 30°C for 24 ± 2 hours incubation. If microorganisms produced gas, the uncertain colonies were considered as coliform and were counted. If tubes were turbid and no gas was produced, suspension was streaked on Mac conkey agar. Coliforms bacteria which formed reddish purple colonies on the medium were counted. Total coliforms number was calculated using the following equation, Eq.2 (ISO, 2004).

\[ \text{Eq.2: Coliforms number (mL)} = \text{total number of colonies (confirmed and uncertain colonies)} \times \text{dilution-1} \times \text{volume-1} \]

3. RESULTS

The experiment which carried out on 118 milk samples according to the Iran’s national standard demonstrated that 11% percent of products were inconsumable. Test results were reported distinctly in table 2.

<table>
<thead>
<tr>
<th>Contamination item</th>
<th>Number of samples out of standard range from 118 samples</th>
<th>% of contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.Coli</td>
<td>3</td>
<td>11.01%</td>
</tr>
<tr>
<td>Coliforms</td>
<td>11</td>
<td>9.32%</td>
</tr>
<tr>
<td>Total count</td>
<td>13</td>
<td>2.54%</td>
</tr>
</tbody>
</table>

4. CONCLUSION

This study investigate contamination level of 118 pasteurized milk sample were collected from different supplier centers of Lorestan province. Counting results of total microbial population, coliforms and E.coli determined health status of milk samples. 11% of samples were tested below the norm and were inconsumable for human. There are many reports up to now reported microbial contamination of dairy products. 61% and 5% of raw milk and pasteurized milk sample were collected in Shahrood city of Iran were contaminated and labeled inconsumable (Arab Amery, 2007). Salari and coworkers found that about 81% of milk sample in Yazd city of Iran satisfied standard criteria (Salari, 2007). Quality Evaluation of raw milk was produced in Kerman province of Iran were near 37.31×106 and 7.47×106 cfu/ml for microbial load in bulk storage and through delivery time (Vahmi, 2003). Microbial analysis of 300 milk and yogurt samples were collected from Dhaka city in Bangladesh showed that all samples had E.coli and Shigella-like species contamination and all pasteurized milk contained total bacterial count of 1.9 × 102 to 2.8 × 103cfu/ml. also it has been stated that microbial competition and low pH are the inhibitory factor for contamination (Rahman, 2008). Another studies detected different contamination in pasteurized milk and suggested controlling critical steps of pasteurization like heating, handling, storage and post.
pasteurization process confronted with bacteria (Rizwanet, 2011; Khan, 2008). A study on 739 pasteurized milk sample carried out in Iran showed 8.68% of products were contaminated higher than standard level (Karimi, 2006). In a distinctive observation indicated that 19.7% of milk, 49% of transporting and 58.4% of selling center weak operation were leaded to E.coli and S.aureus contamination of milk (Sadeghi-Fard, 2006). Pourhassanan and Taravat reported proportion of E.coli, Enterobacter, Klebsiella and S. aureus in raw milk contamination in Malayer city of Iran (Pourhassanan and Taravat, 2011).

Essential oils and extract of Iranian herbal plants have several antioxidant and bioactive compounds which those have antimicrobial effect on any types of bacteria such as gram-negative and gram-positive bacteria. Therefore, they can be used for safeguarding of food and decreased of microbial quality (Bahmani, 2014; 2015; Delfan, 2014; Sarrafchi, 2015; Asadi-Samani, 2014; Saki, 2014; Karamati, 2014; Asadbeygfi, 2014).

The diversity of reports is because of different range of personal hygiene, delivery system quality, maintaining equipment and regards for milking. Beside these factors, season of sampling may affect the subject. According to Iran standard and testing institute, 13 sample from 118 exceeded the norms of microbial load which oriented by improper processing and packaging. Considering the microbial load of contaminated sample it could be recommended that, in the first place raw milk contamination by reason of unhygienic soil, feed, air, water, cow udders, milkers' hands and milking equipment in farms should be protected from hazardous microorganism, because Spore prone microorganism can emerge after pasteurization process and spoil products. In the second place using appropriate equipment for milk handling and storage and staff training can prevent further contaminations in collection centers and factories.

5. ACKNOWLEDGMENT

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REFERENCES


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