Comparison Between the Amount of Penicillin G Residue in Raw and Pasteurized Milk in Iran

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1. Background

The importance of milk and its related products in human diet has been thoroughly realized since early times. Milk is one of the most appropriate and moderate foods including various types of proteins, lipids, minerals (calcium, phosphate and potassium), vitamins (D, A, B2, B12), lactose and the entire amino acids required for the maintenance, growth and development of the body tissues (1). Checking the milk quality and making assurance of its healthiness is of great importance. In order to improve the milk quality and healthiness, it should be free of every physical, biological and chemical contaminations. For instance, different kinds of antibiotics, most typically the Benzylpenicillin (2), which can basically cause numerous problems to the dairy industry (3-5).

Several studies were conducted to determine antibiotic availability in milk and milk products in different countries. Monitoring of the raw and pasteurized milk samples in the United States of Canada and the UK from 1955 and 1959, shown that more than 6.11% of the samples were contaminated with penicillin (6). A study performed in 1958 and 1959 in South Africa showed that 3% of 1200 investigated samples, were contaminated with penicillin (6). A survey in Pennsylvania published in 1959 revealed that 77% of the dairy farmers have not discard treated milk for a 72-hour period (7). The State of Pennsylvania now has a law insisting on testing all milk products once a month for antibiotic residues (8). Afnan and Kashani investigated that among 402 raw and 432 pasteurized milk samples, 22.5% of raw milk samples and 5.2% of pasteurized ones were contaminated with penicillin (9). In another study, Karim and Nawab indicated that over 52% of the milk samples were contaminated by drug residues (10).

If the contaminated Milk with antibiotic residues is used, it will contaminate other dairy products. If the milk is dried, evaporated, or made into ice cream, the antibiot-
Milk antibiotic contamination is an important global problem, thus qualitative and quantitative controls of milk samples are necessary. Consequently, the aim of this study was to monitor antibiotic contamination in milk samples using beta-star test and cylinder plate method.

3. Materials and Methods

3.1. Samples and Reagents

992 milk samples and three replications of each were collected from farms and milk industries in 2011. Seed Agar and Yeast Beel Agar were purchased from HIMEDIA, Sweden. Potassium dihydrogen phosphate and K2HPO4 were purchased from Merck Company, Germany. Penicillinase (β-lactamase) and Penicillin G standard were provided by Sigma, USA. Micrococcus luteus (ATCC 9341) was purchased from IAM, Japan.

3.2. Preparation of Penicillin G Standard Solution

100 μg/mL stock solution of standard Penicillin G was prepared by dissolving 100 μg of the standard in 1 mL of phosphate buffer. Different concentrations of 0.2, 0.1, 0.05, 0.025, 0.0125, 0.0067 and 0.000625 μg/mL were prepared by adding the corresponding amounts of healthy and sterile milk.

3.3. Cylinder Plate Method

In order to perform Cylinder plate test, M. luteus (ATCC 9341) was used. Microbial suspension was prepared as follows: adequate amount of tested microorganism was cultivated on an agar medium in a way that the entire surface of the agar was covered by the microorganism. Then the medium was held at 32-35°C for 18-24 h. Following, the microbial layer on the surface of the agar was transferred to seed agar and was held at 32-35°C for 18-24 h. Optimum inhibition is achieved when only 20% of the light with 650 nm length is passed from the suspension. Anti-bacterial activities of Penicillin G standard solutions and milk samples were investigated through cylinder plate method. Plates were kept at room temperature for 3 h and then at 30°C for 18 h.

3.4. Beta-star Test

The commercially available beta-star test is a receptor assay for rapid detection of β-lactam antibiotic residues
in milk. The test is interpreted by visual comparison of a band with a reference band. Results are classified in to 4 categories; steps 2, 3 and 4 are interpreted as positive. The control samples were prepared according to ISO18330/IDF 188:2003. 0.2 mL of the sample was injected into the vials by the use of the automatic syringe and plastic micropipette. Vials were incubated at 47.5 °C for 3 min and then one dipstick was dipped into every vial. Then, vials were again incubated at the same temperature for 2 min.

3.5. Statistical Analyses

This experiment was carried out in a completely randomized design (CRD) with three replications. Data were analyzed statistically by the Statistical Package for Social Sciences (SPSS) 16.

4. Results

In order to screen the antibiotic contaminated sample, beta-star test was selected. Results of beta-star test are shown in Table 1. As it is shown in Table 1, 23.8% of the raw milk samples and 10.2% of the pasteurized milk samples were estimated to have positive responses. However, beta-star test is responding to all beta lactam antibiotics, the type of antibiotic is not detectable by using this test.

Table 1. Results of the Bata-Star Test of Milk Samples with Three Replications Collected from Farms and Milk Industries in 2011

<table>
<thead>
<tr>
<th>Province</th>
<th>Raw Milk, No. (%)</th>
<th>Pasteurized Milk, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zanjan</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>Tehran</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Kerman</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Gilan</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Mashhad</td>
<td>41</td>
<td>11</td>
</tr>
<tr>
<td>Gorgan</td>
<td>40</td>
<td>10</td>
</tr>
</tbody>
</table>

The result showed that the level of beta lactam antibiotic residues in milk were high in milk in Iran. This report is in accordance with other reports in other countries. In Iran, Movassagh and Karami. showed that 5% of cow raw milk was positive for antibiotics residues (25). Kaya and Filazi reported that in 204 raw milk samples, about 44% was positive for antibiotic residues (26). Khoshkheili et al. (27) showed that of all samples 36.5% were contaminated by beta lactam antibiotic residues in cow raw milk in Pakistan. The prevalence of antimicrobial residues in preprocessed and processed cow milk in Trinidad were studied, and reported that 10.8% of all samples were positive (28). In a study by Shitandi of a total of 1109 milk samples collected from Kenya, 21% was positive for antibiotic residues (29). Ceyhan and Bozkurt (30), in 200 milk samples, 5.5% was positive for antibiotic residues in Ankara region.

Table 3. Amount of Penicillin G in Milk Samples which were Collected from Farms and Milk Industries in 2011 by Cylinder Plat Method

<table>
<thead>
<tr>
<th>Province</th>
<th>Raw Milk</th>
<th>Pasteurized Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zanjan</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Tehran</td>
<td>2.2</td>
<td>10</td>
</tr>
<tr>
<td>Kerman</td>
<td>1.74</td>
<td>15</td>
</tr>
<tr>
<td>Gilan</td>
<td>0.67</td>
<td>10</td>
</tr>
<tr>
<td>Mashhad</td>
<td>1.35</td>
<td>11.21</td>
</tr>
<tr>
<td>Gorgan</td>
<td>0.55</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The result showed that the level of beta lactam antibiotic residues in milk were high in milk in Iran. This report is in accordance with other reports in other countries. In Iran, Movassagh and Karami. showed that 5% of cow raw milk was positive for antibiotics residues (25). Kaya and Filazi reported that in 204 raw milk samples, about 44% was positive for antibiotic residues (26). Khoshkheili et al. (27) showed that of all samples 36.5% were contaminated by beta lactam antibiotic residues in cow raw milk in Pakistan.

5. Discussion

In this study, 23.8% of raw milk samples and 10.2% of pasteurized milk samples were shown the presence of beta lactam antibiotic residues in Iran, which has a very high prevalence for Iran. Table 1 compares the positive, negative, and suspected results of raw and pasteurized milk samples to beta-star test. As it is indicated, while about one fourth of raw milk samples had positive responses to this test, while only one tenth of the pasteurized samples showed positive results. This data demonstrates that pasteurizing milk samples results show more than two folds reduction of betalactam antibiotic levels in samples. Also, beta star kit could detect all kind of beta lactams in milk.

Monitoring of beta-lactam residues of edible tissues and milk is important because of the hypersensitivity of some individuals to these antibiotics and also the emer-
gence of antibiotic-resistant strains of bacteria. In addition to allergic reaction, there are some indications in the scientific literature suggesting that antibiotics can induce cancer and other non-cancerous health hazardous effects. Kosikowski (31) recently collected data on the national incidence of antibiotic residues in milk from January 1 to October 15, 1960—a period of testing and surveillance by dairy and regulatory officials. A survey in Pennsylvania published in 1959 revealed that 77% of the dairy farmers were not discarding treated milk for a 72-hour period (7). The presence of antibiotics in milk has been reported in Canada (32). Starter cultures were inhibited in milk from 7.3% of 344 herds sampled in spring of 1952 that 5.4% of 298 herds expressed similar degrees of inhibition in the summer of the same year. The presence of antibiotics in milk has been reported in Indonesia (32). Starter cultures were inhibited in milk from 7.3% of 344 herds sampled in spring of 1952 and 5.4% of 298 herds expressed similar degrees of inhibition in the summer of the same year.

Since beta-star method is responding to all beta lactam antibiotics, it would be better to select a method which is specific to Penicillin G. Though, cylinder plate method is a time-consuming, expensive and difficult test, compared with beta-star test. Therefore, it was necessary to screen all samples with beta-star test before performing cylinder plat test for samples which had positive response to beta-star test. Thus, cylinder method was utilized. On top of that, beta-star is a qualitative method which is only capable to reveal the presence of the beta lactam antibiotics and cannot measure the exact amount of contamination. On the other hand, cylinder plate is a quantitative technique to measure the amount of contamination that makes it a suitable method for determination of Penicillin G in milk samples. Table 2 shows the responses of raw and pasteurized milk samples to cylinder plate method. Around 11% of the raw samples showed positive responses to this method, while according to cylinder plate method only 1.5% of the pasteurized samples were contaminated to Penicillin G antibiotic, meaning that the process of pasteurization resulted in a about 8-times reduction of Penicillin G contamination within milk samples.

In comparison with the results of Table 1, the percentage of contaminations are lower, since beta-star test responses to all beta lactam antibiotics, including Penicillin G, whereas cylinder plate method just measures the levels of PG in milk samples. As it is demonstrated in Table 3, amongst raw milk samples Tehran showed the maximum average concentration of 2.2 µg.mL⁻¹, and Zanjan had the minimum average concentration of 0.5 µg.mL⁻¹. Most of the milk samples did not have antibiotic contamination after pasteurization. This affair is only exceeded in Mashhad which showed an average concentration of 0.21 µg.mL⁻¹.

Results show the amount of contamination is dramatically reduced through the process of pasteurization. Beta-lactam ring within the structure of beta lactam antibiotics is highly heat-sensitive, and warming up the milk samples till 80 °C during the process of pasteurization causes this ring to be degraded. Since this ring plays a major role in the biological activities of beta lactam antibiotics, it would be suggested that pasteurization process removes antibiotic residues through degrading this ring.

Since milk and dairy products are considered as a crucial part in nutrition pyramid, controlling the quality of the antibiotics would be an essential step before releasing those products in marketplaces. One of the major concerns in this field is monitoring the presence of antibiotic residues in milk, because the contamination is available in other dairy products as well. Among different antibiotics which are utilized as medicine to treat mastitis, Penicillin G is the most common one. In this study two types of experiments were performed to measure the amount of Penicillin G residues in milk. Results revealed that cylinder-plate method accompanied by beta-star test can be considered as an appropriate sensitive and selective method for the routine control of milk and dairy products.

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Authors' Contribution

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References


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