Detection Beta-lactam residues in imported and local dairy products in Mosul city

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Abstract
Two hundred and forty samples of milk products were examined for ß-Lactam antibiotics residues by using a quantitative technique (Sandwich-ELISA). Basically, the study comprised three different dairy farm animals (buffalo, cow, and sheep), the samples were collected from Mosul city farms, and compared to imported dairy products from exotic sources. Apparent ß-Lactam residues were found most of the milk products, considerably local and imported products. In terms of local milk products were 50% of cow, 60% of sheep, and 20% of buffalo mostly free from antibiotic residue. In contrast, imported milk products were 90% of cow, 70% of sheep, and 10% of buffalo, also free from antibiotic residue and suitable for human consumption. However, there were many samples of milk products contaminated with ß-Lactam residue, in particular, buffalo products were high concentrations of ß-Lactam compounds, regardless of whether local and imported products. With respect to cow products, 50% of local samples compared to 10% of imported samples were contaminated with ß-Lactam residues. Whereas in sheep products, 40% of local products compared to 30% of imported products were high concentration of antibiotic residues. Surprisingly, for all samples of this study, it has been noticed within the same product, the concentration of ß-Lactam was high level in cheese and cream compared to milk raw, however, in yogurt were decreased sharply 45% compared to cream, cheese, and milk respectively.

Keys words Public health, Antibiotic residues, ELISA, Milk products)

1. Introduction
The contamination of milk products with antibiotic residues is a considerable problem in public health and the dairy industry. Usually, antibiotic residues presence milk products from lactating animals have treated to antibiotics, in particular, that suffering from mastitis or other diseases and sometimes antibiotics are added to the milk to prevent bacterial proliferation [1, 2]. ß-Lactam antibiotics commonly are widely used in pharmaceutical treatment in veterinary and human medicine, which have many kinds including penicillins, cephalosporins, carbapenems, monobactams, and penems [3]. In general, during animals taking antibiotics leads to the beginning a relative improvement in animal health, however, each ß-Lactam antibiotic has withdrawal period from the body depend on the kinetics of chemical compounds, which extends from 7-14 days [4]. At this point, ß-Lactam antibiotics spreading throughout the whole body tissues of the animal including the mammary gland, and excreted it into the milk resulting in health problems in humans, also increasing the resistance of bacteria against antibiotics [5]. At the environmental aspect, there is a high percentage of antibiotics every moment have excreted by animals and humans via urine and faeces into wastewater sewage, resulting in environmental issue problems [6]. The Enzyme-Linked Immunosorbenent Assay (ELISA) has considered the most accurate technique for detecting antibiotics in different liquids by coupled with specific antibodies [7]. In contrast, there are many methods have been used for the detection of ß-Lactams in milk products, using High-performance liquid chromatography, UV detection, and other techniques [8]. In this study, we examined local versus imported milk products by using a sandwich ELISA for investigating whether dairy products are contaminated with ß-Lactam antibiotics or not, and suitable for human consumption.

2. Material and Methods
A total of 240 milk products samples were collected that included (milk, yoghurt, cream and cheese) from over a one year period divided into 120 samples from imported products and 120 samples from around Mosul city farms. All samples were kept at 4°C and examined within 12 hours. Then, all samples tested for ß-Lactam antibiotic residues using employs the quantitative enzyme immunoassay technique. The ELISA method was subjected according to the manufacturer’s instruction (Mesline co. EKHU-0984). The test principle consists of a micro-plate, that has been pre-coated with a specific ß-Lactam
antibody. After adding all samples into the micro-wells the plate incubation an hour which allows conjugate Ag-Ab reaction, then washing it in order to remove the uncombined enzyme and add chromogen solution, and finally add stop solution to each well and the color will be changed from blue to yellow for measurement optical density (O.D.) via spectrophotometrically at a wavelength of 450 nm. All results samples have obtained and blotted within the standard curve for determination each sample accurately. The statistical analysis of data has examined by (R) test.

3. Results
The study was distributed to three different types of samples with two groups (local and imported milk products). The ELISA results showed that out of 240 samples. Table 1 and 2 demonstrates the level of β-Lactam antibiotics concentrations of local and imported milk products in different animal (buffalo, cow, and sheep). In terms of buffalo samples, eight local farms were positive of level contamination of their products, however, imported products were nine out of ten companies also contaminated too in Figure 1. In cow milk products, five out of ten local farms were present antibiotics residues in their products, compared to imported products demonstrating the Significant decrease in residue levels of concentrations, which were one out of ten companies in Figure 2. With respect sheep products have found mostly the same levels of antibiotics contamination, four out to ten from local farms, while imported products were three out to ten companies in Figure 3. Interestingly, we found in all milk products shows the highest detection of antibiotic residues was in cheese, cream, milk, and yogurt respectively.

Table 1. A survey of study to detect β-Lactam antibiotics residues in local milk products of different dairy animals in ten different farms in Mosul city

| Animal species for local products | Farms |                   |                   |                   |                   |                   |                   |                   |                   |           |
|----------------------------------|-------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-----------|
|                                  | 1     | 2                | 3                | 4                | 5                | 6                | 7                | 8                | 9                | 10        |
| Buffalo β-Lactam antibiotics concentration (ng/ml) |       |                   |                   |                   |                   |                   |                   |                   |                   |           |
| Milk                             | 167.8 | 40.1             | 120.2            | 60.5             | 0.9              | 0                | 22.3             | 19.8             | 18.7             | 78.9      |
| Yogurt                           | 114.5 | 33.4             | 88.1             | 30.5             | 0.3              | 0                | 15.6             | 10.9             | 20.5             | 45.6      |
| Cream                            | 178.9 | 50.5             | 130              | 58.3             | 1.1              | 0                | 29.8             | 22.6             | 30.9             | 80.4      |
| Cheese                           | 180.9 | 56.7             | 0.29             | 62.8             | 3.4              | 0                | 30.8             | 30               | 35.6             | 90.9      |
| Cow β-Lactam antibiotics concentration (ng/ml) |       |                   |                   |                   |                   |                   |                   |                   |                   |           |
| Milk                             | 75.6  | 0.15             | 0.25             | 60.5             | 0.9              | 0                | 78.9             | 16.3             | 0.12             | 155.6     |
| Yogurt                           | 15    | 0                | 0.1              | 30.5             | 0                | 28               | 4.02             | 0.01             | 45.6             | 0         |
| Cream                            | 60.9  | 0.125            | 0.21             | 58.3             | 0                | 76.1             | 19.2             | 0.17             | 101.2            | 0.18      |
| Cheese                           | 61.6  | 0.2              | 0.29             | 62.8             | 0                | 82               | 20.4             | 0.13             | 156.9            | 0.16      |
| Sheep β-Lactam antibiotics concentration (ng/ml) |       |                   |                   |                   |                   |                   |                   |                   |                   |           |
| Milk                             | 0.19  | 0                | 101.6            | 0.54             | 88.9             | 12.56            | 0.7              | 0                | 34.4             | 0.9       |
| Yogurt                           | 0.01  | 0                | 56.6             | 0.44             | 40.8             | 7.9              | 0.4              | 0                | 24.3             | 0.44      |
| Cream                            | 0.2   | 0                | 120.2            | 0.67             | 97.8             | 12.9             | 0.9              | 0                | 38.9             | 1.2       |
| Cheese                           | 0.23  | 0                | 112.9            | 0.77             | 95.6             | 13.01            | 1.32             | 0                | 40.8             | 1.9       |
Table 2. A survey of study to detect β-Lactam antibiotics residues in imported milk products of different dairy animals in ten different companies

| Animal species for imported products | Dairy companies |  |  |  |  |  |  |  |  |  |
|-------------------------------------|----------------|---|---|---|---|---|---|---|---|---|
| Buffalo                            | β-Lactam antibiotics concentration (ng/ml) |  |  |  |  |  |  |  |  |  |
| Milk                               | 86.7 9 171.16 0.275 74.25 17.93 66.55 | 24.53 21.78 20.57 |  |  |  |  |  |  |  |  |
| Yogurt                             | 50.1 6 16.5 50.237 0.11 55.55 4.422 33.55 | 17.16 11.99 22.55 |  |  |  |  |  |  |  |  |
| Cream                              | 88.4 4 66.9 9 111.32 0.231 88.33 21.12 64.13 | 32.78 24.86 33.99 |  |  |  |  |  |  |  |  |
| Cheese                             | 99.9 9 67.7 6 172.59 0.319 75.75 7 22.48 4 69.15 | 33.88 33 39.16 |  |  |  |  |  |  |  |  |
| Cow                                | β-Lactam antibiotics concentration (ng/ml) |  |  |  |  |  |  |  |  |  |
| Milk                               | 0.285 0.105 0.225 0.375 0.4125 24.45 0.375 0.15 | 0.015 0 0 0 0.15 0.165 6.03 0.15 0.04 |  |  |  |  |  |  |  |  |
| Yogurt                             | 0.3 1.35 0.187 0.315 0.3465 28.8 0.315 0.27 | 0.345 0 1.98 0 0.3 0.435 0.4785 30.66 0.435 0.25 |  |  |  |  |  |  |  |  |
| Cream                              | 0.207 0.81 91.08 0.16 17.28 0.15 0.189 52.47 0.18 0 | 0.207 1.19 141.2 0.15 18.39 0.11 0.261 56.58 0.207 0 |  |  |  |  |  |  |  |  |
| Sheep                              | β-Lactam antibiotics concentration (ng/ml) |  |  |  |  |  |  |  |  |  |
| Milk                               | 0.247 0.63 140 0.09 14.6 0.108 0.225 54.45 0.171 0 | 0.099 0.36 41.1 0 3.61 0.01 0.09 27.45 0.009 0 |  |  |  |  |  |  |  |  |
| Yogurt                             | 0.207 0.81 91.08 0.16 17.28 0.15 0.189 52.47 0.18 0 | 0.207 1.19 141.2 0.15 18.39 0.11 0.261 56.58 0.207 0 |  |  |  |  |  |  |  |  |
| Cream                              | 0.207 1.19 141.2 0.15 18.39 0.11 0.261 56.58 0.207 0 | 0.207 1.19 141.2 0.15 18.39 0.11 0.261 56.58 0.207 0 |  |  |  |  |  |  |  |  |

Figure 1. Method for detection of β-Lactam antibiotics concentration in buffalo local products compared to imported products was analyzed by ELISA. Noticeably, the experiment was repeated triplicate.
Figure 2. Method for detection of β-Lactam antibiotics concentration in cow local products compared to imported products was analyzed by ELISA. Noticeably, the experiment was repeated triplicate.

Figure 3. Method for detection of β-Lactam antibiotics concentration in sheep local products compared to imported products was analyzed by ELISA. Noticeably, the experiment was repeated triplicate.

4. Discussion
Presence β-Lactam antibiotic residue diagnosis was carried out serologically by using enzyme-linked immunosorbent assay (ELISA). There are many techniques had been used for detecting antibiotic residues in milk products like the four-plate test, Solid-phase extraction (SPE), High-performance liquid chromatography (HPLC), and others [9-11]. However, quantitative ELISA has considered an accurate technique for determining antibiotic residues in milk products and cell tissue [11]. Because in this case using specific antibodies that directly react with b-lactam particles that is undergo the principle of Ag-Ab reaction and give us the results precisely [12]. Moreover, our data revealed that the sensitivity of Sandwich-ELISA for detection the minimum amount of antibiotic residues approximately 0.1 ng/ml. As shown in this data, the high percentage of contamination of β-lactam were in buffalo products and the results are compatible other researchers [13, 14]. In contrast, the percentage of antibiotic residues imported cow milk products was decreased (10%) compared with local products (50%), because recurrent mastitis and continuous treatment [15]. With respects of sheep, the results was found 35% of milk products that contaminated with antibiotic residues whether imported or local sources, and the data obtained has completely different from previous study was found 90% of milk products contaminated with antibiotic residues [16]. In summary, there are special precautions for using antibiotics in dairy animals, that prohibited using their products during the treatment period, also, each antibiotic has a withdrawal period of the medicine from the body until reaching a zero-tolerance extend from 7-14 days depending on the antibiotic compound structure [17]. Our results indicate that most of the farms and imported products
uses antibiotics randomly and there are must be strict restrictions that dealing with contaminated products and prevent being used.

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