Detection of Residual Antibiotics in Fresh Cow Milk

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Abstract

The presence of commonly used antibiotics, namely oxytetracycline, benzylpenicilin, streptomycin sulfate, chloramphenicol, ampicilin, sulfonamide and rifampicin in 5 collected cow milk samples were determined qualitatively and quantitatively. Except oxytetracycline, no antibiotics were found in samples. The amount of oxytetracycline in milk samples were found 1800 ng/ml. 2700 ng/ml, 2800 ng/ml, 1700 ng/ml and 2000 ng/ml in samples 1 – 5, respectively which are much higher than WHO and FDA recommended level.

Keywords: detection of antibiotic, oxytetracycline, spectroscopic analysis, cow milk.

Introduction

The widespread use of antibiotics in dairy cattle management may result in the presence of antibiotic residue in milk. Consumption of milk with such antibiotic residue levels by humans predisposes them to serious health effects. Antibiotics given to cows commonly are penicillin, oxytetracycline, sulfadiadine, metronidazole, chloramphanicol, cephalosporin, streptomycin, rifampicin etc. Among them the antibiotics which are commonly excreted through milk are oxytetracycline, chloramphenicol and streptomycin.

To prevent any harmful health effects to consumers, Food and Agricultural organization (FAO), European Union (EU) and Japan have established the maximum residual limit (MRL) of oxytetracycline in milk at 100 ng/ml (Naoto, 1999) and the 'safe levels' set by the US Food and Drug Administration are 30 ng/ml for oxytetracycline, 30 ng/ml for chlortetracycline and 80ng/ml for tetracycline (Popadoyannis *et al*., 2000). Also the WHO recommends a maximum allowable level of 10 0ng/ml for oxytetracycline (Boatto *et al*., 1999).

Antibiotics are used to treat bacterial infections. However-improper use of different classes of antibiotics causes bacterial resistance against infectious diseases both human and animals. We know that among all the antibiotics, some are execrated through mammilary gland both human and animals. Widespread use of antibiotics in dairy cattle management may result in the presence of antibiotic residue in milk. Consumption of milk with such antibiotic residue levels by humans predisposes them to serious health effects. In this study we try to find out which antibiotics are usually excreted through cow milk in what amount, since presence of antibiotics in milk for the safety of consumers.

Materials and Methods

***Milk samples collection*:** Milk sample were collected from retail shops, milk centers and farmers of Chittagong city. Sampling procedures for the milk centers and farmers was done by convenience since there are no specific centers in some areas and the distribution of farmers are not normal. For the processed milk from milk industries, samples were collected basing on their sources and batch numbers.

***Reagents used in chemical test*:** Oxytetracycline, benzylpenicilin, streptomycin sulfate,

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chloramphenicol, ampicilin, sulfonamide and rifampicin available in local market and which are used in veterinary are taken as reference. $AgNO_3$ (40 g/l) TS, ammonia (100 g/l) TS, sulfuric acid (1760 g/l) TS, sodium hydroxide (-80 g/l), potassium permanganate (-10 g/l) TS, hydrochloric acid (-250 g/l) TS, ferric chloride (-25 g/l) TS, liquefied phenol, potassium hydroxide, formaldehyde TS, Copper (II) sulfate (-160 g/l) TS, 1% glacial acetic acid solution and trichloroacetic acid (TCA) 10%. Double distilled water was prepared in our lab.

***Deproteinization of collected milk*:** Milk samples and standard spiked milk were subjected to deproteinizing by a chemical procedure using TCA. 3ml of milk sample were placed into a 5ml falcon test tube and shaken vigorously, then incubate 10 minutes at 4ºC. After 10 minutes the test tubes were placed for centrifugation at 10000 rpm for 15 minutes. Two distinct layers were formed – one is a clear supernatant and other is precipitation. The supernatant were collected by decantation and filtration. Finally supernatant were stored at 4ºC for experiment.

***Qualitative analysis for the detection of antibiotics*:** Qualitative analysis of milk samples for the detection of antibiotics namely-oxytetracycline, benzyl penicillin, streptomycin sulfate, ampicilin, chloramphenicol, sulfadimidine, rifampicin were done by chemical test and the procedures were done according to World Health Organization's Basic tests for pharmaceutical Dosage Forms.

***Quantitative analysis for the detection of oxytetracycline*:** Standard calibration curve was obtained by preparing aqueous solutions of different concentration of standard oxytetracycline and measured the absorbance at 354 nm. Then the supernatants of milk samples were taken and measured the absorbance at 354 nm by "Helos–Thermoelectron spectrophotometry to determine the amount of oxytetracycline.

Spectroscopic analysis of milk sample for Oxytetracycline: Renamycin the oxytetracycline (OTC) preparation of Reneta Pharmaceutical Limited was taken as standard product for ultraviolet spectrophotometric analysis, which contains 50 mg OTC in each ml. From this standard preparation, solution of different concentrations were prepared and the absorbance was obtained at 354 nm (room temperature, ie 24ºC). Standard calibration curve was obtained by preparing aqueous solutions of different concentration of standard oxytetracycline and measured the absorbance at 354 nm. Then the supernatants of milk samples were taken and measured the absorbance at 354 nm to determine the amount of oxytetracycline.

Results and Discussion

Chemical assay for qualitative analysis

The chemical assays were performed on different collected samples to determine the presence of various antibiotics in milk and the results are given below

Table 1: Results of chemical assay for qualitative analysis

Milk Sample	A1	A2	A3	A4	A5	A6	A7
Sample-1	+	-	-	-	-	-	-
Sample-2	+	-	-	-	-	-	-
Sample-3	+	-	-	-	-	-	-
Sample-4	+	-	-	-	-	-	-
Sample-5	+	-	-	-	-	-	-

A1: oxytetracycline; **A2**: benzylpenicilin; **A3**: streptomycin sulfate; **A4**: chloramphenicol; **A5**: ampicilin; **A6**: sulfonamide; **A7**: rifampicin.

From the above table it was found that all milk samples were contain oxytetracycline and other antibiotics were not found. We performed positive and negative control test for all antibiotics. Incase of oxytetracycline we observed similar results both in milk sample tests and positive control test.

Qualitative determination of oxytetracycline

The concentrations of oxytetracycline were calculated from the calibration curve. The calculated results are given below in table 2.

Table 2: Concentration of oxytetracycline in different milk samples

Milk sample	Concentration (ng/ml)
Sample-1	1800
Sample-2	2700
Sample-3	2800
Sample-4	1700
Sample-5	2000

It was reveled from table 2 that the entire milk samples contain oxytetracycline more than the maximum allowable level according to FDA and WHO.

Conclusion

Presence of antibiotics namely oxytetracycline, benzylpenicilin, streptomycin sulfate, chloramphenicol, ampicilin, sulfonamide and rifampicin in collected cow milk samples were determined by using chemical assay. Other than oxytetracycline, no antibiotics were found in the samples. By quantitative analysis we found 1800ng/ml, 2700 ng/ml, 2800 ng/ml, 1700 ng/ml and 2000 ng/ml of Oxytetracycline in sample 1 – 5 respectively. These amounts are much higher than the officially recommended amount. Since we have got residual oxytetracycline in milk and we know that milk contained different type of pathogenic and non-pathogenic microbes, hence these microbes will be the resistant in such milk.

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