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Antibiotic Residues Detected in Commercial Cow's Milk

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Summary

Cow's milk is an important food containing vital nutrients for both children and adults. Chemical residues and antimicrobials in milk may be a concern for consumers. In this study we tested the hypothesis that cow milk sold for human consumption contains antibiotic residues. The general approach was to use the Delvo P Test to detect antibiotics in ten different milk samples. According to the results obtained, nine out of the ten analyzed milk samples sold commercially in Greensboro, NC contained antibiotics at five parts per billion or higher. The findings shed light onto the methods for ensuring the safety of milk. The antibiotics in the milk might pose a risk for human health because antibiotic residues might trigger antibiotic resistance in human gut bacteria.

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Introduction

As the world's population increases, cow's milk, consumed by humans for millennia, is becoming an even more important source of vital nutrients, including protein, calcium, and vitamins. Over the past two centuries, the amount of milk produced per cow has dramatically increased because of better genetic selection, better animal management, and improved animal health (1). One of the major diseases that impairs milk production is an infection of the mammary glands, called mastitis, (2) caused by bacteria, including species of *Pseudomonas*, *Klebsiella* and *Staphylococcus* (3). Some of these bacteria also cause disease in humans, such as the respiratory illness pneumonia and methicillin-resistant *Staphylococcus aureus* (MRSA) infection, which seriously threatens human health (4). Mastitis not only makes the cows sick, but it also decreases the quantity and quality of the milk. In cows, the infection causes inflammation of the mammary gland, and toxins produced by bacteria damage the tissue. The udder may become swollen, leukocytes or white blood cells in the milk increase, and milk production is negatively affected. If left untreated, the cows may lose their appetite, have fever and depression, and even die (5). To treat mastitis infections, antibiotics are administered at the clinically relevant concentrations for the correct period of time. During this time period, it is recommended that milk from these cows be discarded and properly destroyed.

Antibacterials are antimicrobial chemicals produced by bacteria to inhibit or kill other microorganisms. In the case of the treatment of mastitis, the most commonly used antibiotics are penicillin, ampicillin, flunixin, tetracycline, sulfonamides, pirlimycin, novobiocin, sulfadimethoxine, florfenicol, cephapirin, and combinations of lincosamides and macrolides (6). The major concern in the treatment of microbial infections and the administration of antibiotics to animals is that the bacteria can develop resistance against the antibiotics being used to combat the diseases.

Bacteria are versatile organisms, and they can develop resistance against antibiotics in multiple ways. For example, bacteria may develop systems to modify the specific antibiotic target within the bacteria. They may pump the antibiotic out of the cell, or they may cleave the antibiotic. Incorrect use of antibiotics, such as the wrong dose or usage for the wrong period of time, can induce bacteria to develop molecular and cellular modifications to adapt to surviving in the presence of antibiotics (7). The alarming increase of antibiotic resistance among bacteria is a huge problem for both veterinary and human medicine.

When cows afflicted with mastitis are treated with antibiotics, it takes several days to first treat the infection and then for antibiotics to clear out of the cow's system. If dairy producers collect milk from these infected cows immediately after the antibiotic treatment, the collected milk samples are expected to contain both the pathogenic bacteria and the antibiotic residues. Any residues from these infected cows will contaminate the milk collected from other cows in the herd if all the milk samples are pooled into one tank. There is an urgent need for studies on this to determine the occurrence and prevalence of antibiotics in the milk consumed by humans. This need justifies this current study, in which, 10 commercially sold milk samples from the Greensboro, NC area were tested for the presence of antibiotic residues. It was

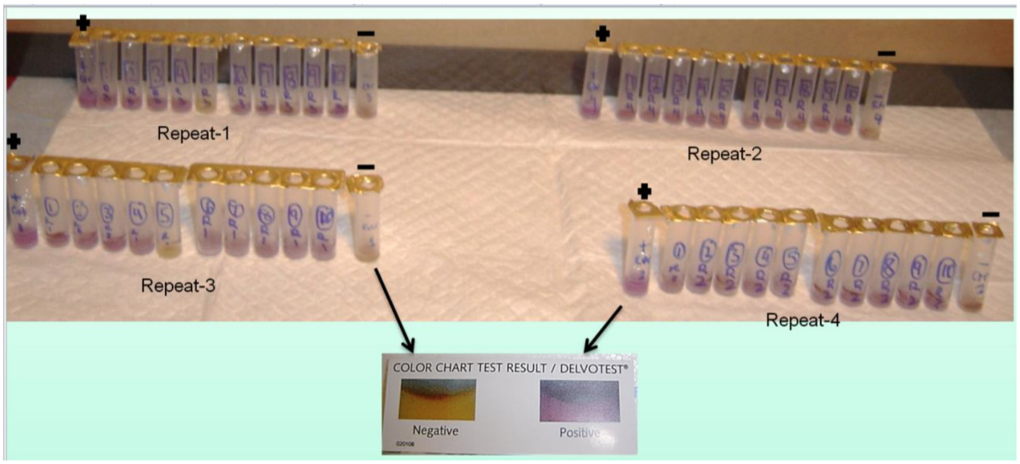

Figure 1: Ten different milk samples were analyzed for the presence of antibiotic residues using the Delvo P Test. The experiments were repeated four times, and a negative and a positive control were included in each repeat. The results showed that all of the milk samples, except one (#5 with orange/yellow color), contained antibiotics at a concentration of at least five parts per billion (purple color).

hypothesized that a majority of these samples would contain antibiotics.

Results

In order to test for the presence of antibiotic residues in 10 milk samples from the Greensboro, NC area, the Delvo P Test was performed on each experimental sample. The Delvo P Test is a colorimetric test used to detect a broad range of antibiotics in milk at 4 parts per billion (ppb); for penicillin the sensitivity is 0.003-0.004 IU/ml. This highly sensitive test utilizes a nutrient substrate; upon mixing it with 0.1 ml milk and incubating in a tube containing gel at 64 ^{o}C for 2.5 hours, the color stays purple if the milk contains antibiotics or changes from purple to orange if it is negative (8,9). The test was also performed on a negative control sample, known to not contain antibiotics, and a positive control sample, known to contain a detectable level of antibiotic residues. Careful analysis of the changes in color in each tube indicated that there was only one tube from the ten experimental samples that showed any orange/yellow color, indicating a negative result. The color of the remaining nine milk samples was purple, indicating a positive result. The negative control was orange/yellow color, and the positive was purple (**Figure 1**). The results demonstrated that 90% of the milk sampled contained antibiotic residues. We have high confidence in these results as consistent results were obtained in four independent replicates.

Discussion

The Delvo P Test used in this research has a sensitivity of five parts per billion (for any of tetracycline, penicillin, ampicillin, amoxicillin, cephapirin, and cefitiofur), which means that all nine of the milk samples contained antibiotic residues at five parts per billion or higher. Clearly, the levels of antibiotics in these milk samples may be higher, but upper levels were not studied. In the future studies, more sophisticated tools capable of analyzing milk samples and identifying wide variety of antibiotics and their chemical properties can be used. For example, chemical tests exist that can determine specific antibiotic residues. In addition, high throughput methods such as mass spectrometry and chromatography could be used to measure precise levels of specific antibiotics. Factors that can enable the uses of these techniques will include feasibility factors such as the cost and availability of such equipment and tests as well as expertise in data analyses.

The United States Department of Food and Drug Administration has extensive regulations about chemical residues in cow milk so that the consumers will have safe milk to consume. Acceptable levels of antibiotics in the milk depend on the types of antibiotics and are regulated by the Food and Drug Administration (10). For example, the acceptable levels for amoxicillin, ampicillin, ceftiofur, cephapirin, cloxacillin, penicillin, are 10 parts per billion (ppb), 10 ppb, 100 ppb, 20 ppb, 10 ppb, and 5 ppb, respectively. Antibiotic residues in the milk are measured using different Food and Drug administration–approved tests or assays, such as the Charm B Tablet Disk Assay, Delvotest P test, and New Snap Beta lactam test (11). The extent of commercial milk samples containing antibiotics depends on, among others, the how diligently and precisely dairy producers measure the antibiotics from each cow that is under antibiotics (and so do not pool the milk that contains antibiotics into the main milk tank), and how widespread the distribution systems for the milk samples are.

Antibiotics are used for the prevention and treatment of diseases, such as reproductive diseases and mastitis in dairy cows. This practice has resulted in healthy cows that produce healthy and nutritious milk, which is vital for human health, too. On the other hand, the use of intramammary antibiotics in treatment of mastitis and waiting shorter than required time periods before milk collection are the major reasons for the presence of antibiotic residues in milk (12, 13). Prevention and treatment of mastitis in cows is important for both human and cow health. The most common method of pasteurizing milk is by heating milk to 72 ºC for 15 seconds and repeating this process several times. Pasteurization inhibits actively growing bacteria, but it does not kill all of the bacteria in the milk, and so pasteurized milk can contain bacteria. To minimize bacteria in milk, mastitis should be prevented and treated, and milk must be collected in clean parlors (14). Indeed, certain infections in humans were shown to be milk-born. For example, *Streptoccus agalactiae* found in milk has been shown to cause infections in humans (15).

Antibiotic residues in the milk may pose two potential risks to humans: 1) allergic reactions among people who are hypersensitive to antibiotics (such as penicillin), and 2) the emergence of antibiotic resistant bacteria. Information on how the consumption of milk containing low levels of antibiotics changes the dynamics of genetics and physiology in the gut bacteria has been elusive due to lack of research on this specific topic. Bacteria can develop remarkable mechanisms of resistance against antibiotics (15). Low levels of antibiotics enable bacteria to develop resistance against that antibiotic. This is a common mechanism in antibiotic-producing bacteria such as Streptomyces fradiae, where low levels of the antibiotic tylosin induce expression of genes encoding resistance mechanisms (16). According to the Centers for Disease Control and Prevention, humans get antibiotic-resistant bacteria through a variety of ways. For example, when people take antibiotics, the gut bacteria develop resistance against the antibiotics ingested (4). Thus, antibiotics should be taken for therapeutic purposes at the recommended dose and duration.

In conclusion, the science based considerations coming from the problem tackled in this study are as following: 1. Milk samples from cows that are treated against mastitis infection should be discarded and destroyed properly. 2. Dairy producers should test for the presence of any antibiotic residues in the milk using a sensitive test such as Delvo P Test to ensure that milk samples pooled do not cross contaminate. 3. Consumers should be informed by this study and become aware of this potential problem as educated members of society.

Materials and Methods

Collection of Milk Samples

Ten unexpired milk samples in bottled containers were obtained from ten different suppliers in Greensboro, North Carolina, United States of America.

Delvo P Test

The Delvo P Test kit was purchased from The Jeffers Farms (Kingsley, PA, USA), and the experiments were performed according to the manufacturer's instructions (7).Briefly, ten tubes containing agar with indicator were set up. Nutrient tablets were then placed into each of them, followed by the addition of 0.1 ml milk samples from each of the ten samples. For the positive and negative controls, we set up control milk solutions with and without antibiotics respectively. The tubes were sealed and incubated at 64 ºC for 2.5 hours. The color changes in each tube, including the positive and negative controls, were recorded. Orange/yellow and purple colors indicated negative and positive results, respectively. The experiments were repeated four times, along with the controls. The changes in the color of the agar in each tube were assessed by three people for all four replicates.

The negative control (milk powder containing no antibiotic) was purchased from the manufacturer, and we followed the manufacturer's guidelines. That is, the negative controls were treated the same way as the experimental samples except that instead of the milk samples, the negative control from the manufacturer was used. The positive control containing the antibiotics was purchased and we followed the manufacturer's recommendations. In this case, the positive control was used in place of the milk samples.

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