A Research Note

Detection of Beta-Lactam Antibiotics in Bulk Tank Milk

MARK MITCHELL, BOB BODKIN, and JIM MARTIN

Ontario Ministry of Agriculture, Food and Rural Affairs, Agricultural and Food Laboratory Services Branch, Agriculture and Food Laboratory Services Centre, Guelph, Ontario, Canada, N1H 8J7

(MS# 94-102: Received 25 April 1994/ Accepted 8 August 1994)

ABSTRACT

A total of 100 raw bulk tank milk samples were tested using both the Delvotest-P agar diffusion test and the Delvo-X-Press enzyme linked immunosorbent assay (ELISA) test for beta-lactam antibiotics. Samples were spiked with various levels of different beta-lactams and detection levels were compared to the manufacturer's reported sensitivities and safe levels currently published for raw milk in Canada's Food and Drugs Regulations. In all cases, both tests met or exceeded the reported test sensitivities and all currently published safe levels, with the exception of cloxacillin on the Delvo-X-Press test only. Current safe levels for cloxacillin are 30 ng/ml; this test only detected levels of 70 ng/ml, despite the manufacturer's claim of 50 ng/ml. No false-positive or -negative samples were found using either test method. Both tests were easy to perform, but the Delvo-X-Press required more technical ability. In view of the decreased detection time offered by the Delvo-X-Press (10 min) (Delvotest-P, 2.5 h) and the test's simplicity, this test should be considered as a screening test on the dairy loading dock before milk is accepted into the plant. In addition, the Delvo-X-Press offers an increased shelf life compared with other ELISA tests, because of the separate packaging for test reagents, which is certainly advantageous for infrequent testing.

Key words: Milk, beta-lactam antibiotics, Delvotest-P, Delvo-X-Press test

Antibiotic therapy has been used in food-animal health management for the past 40 years. Antibiotics are administered to dairy cattle by several different routes: oral, intravenous, subcutaneous, intramuscular, and intra-uterine. Unfortunately, all of these routes can lead to antibiotic residues in the milk (12). The presence of antibiotic residues in milk is undesirable for many reasons. Hypersensitivity reactions in the consumer population, development of resistant pathogenic organisms, inhibition of starter cultures, and poor consumer acceptance are the most commonly cited reasons.

A study of antimicrobial drug use in Michigan dairy herds showed that the most commonly used preparations in order of increasing frequency were the beta-lactams (penicillins and cephalosporins) tetracyclines, aminoglycosides, and sulfonamides. The researchers reported that these drugs were used in over 80% of treatments (10). The most commonly used antibiotic preparations currently licensed for use in lactating dairy cattle in Canada usually contain at least one member of the beta-lactam class of antibiotics, and this class by far has been responsible for most of the contamination found in the Ontario milk supply (1, 3, 13).

To protect the Canadian consumer, safe limits have been established on residual amounts of antibiotics in milk in the form of maximum residue levels (MRLs), also known as tolerance levels (see Table 1) (2). Dosages and milk-withholding times have been established for all licensed products to provide assurance that unsafe levels of residues will not be found in milk destined for human consumption (3).

Test methods have traditionally been based on detection of growth inhibition of a test organism and often require up to 3 h to complete (6, 12). Such testing times have not been practical for the release of tanker-truck milk at the dairy receiving dock. Therefore, much emphasis has been placed in recent years on the development of rapid-detection methods for antibiotics in milk. In the 1970s and 1980s many assay systems were developed, such as radioimmunoassays, enzyme inhibition tests, and enzyme-linked immunosorbent assay (ELISA) procedures, which offer a considerable decrease in test time (4, 5, 8, 11, 12, 14).

This study was conducted to compare the ELISA-based Delvo-X-Press test with the classical microbial inhibition Delvotest-P in regard to ease of use and detection levels of various beta-lactam antibiotics, with reference to Canada's MRLs.

MATERIALS AND METHODS

A total of 100 raw-milk samples were collected from Ontario farm bulk tanks over a 2-week period. All samples were held at 4°C and tested within 48 h. All samples screened negative for antibiotic residues using our laboratory's screening program prior to being used in the trials. The screening method involves initial testing of all samples with a microbial inhibition Delvotest-P test only. Current safe levels for cloxacillin are 30 ng/ml; this test only detected levels of 70 ng/ml, despite the manufacturer's claim of 50 ng/ml. No false-positive or -negative samples were found using either test method. Both tests were easy to perform, but the Delvo-X-Press required more technical ability. In view of the decreased detection time offered by the Delvo-X-Press (10 min) (Delvotest-P, 2.5 h) and the test's simplicity, this test should be considered as a screening test on the dairy loading dock before milk is accepted into the plant. In addition, the Delvo-X-Press offers an increased shelf life compared with other ELISA tests, because of the separate packaging for test reagents, which is certainly advantageous for infrequent testing.

Key words: Milk, beta-lactam antibiotics, Delvotest-P, Delvo-X-Press test

Antibiotic therapy has been used in food-animal health management for the past 40 years. Antibiotics are administered to dairy cattle by several different routes: oral, intravenous, subcutaneous, intramuscular, and intra-uterine. Unfortunately, all of these routes can lead to antibiotic residues in the milk (12). The presence of antibiotic residues in milk is undesirable for many reasons. Hypersensitivity reactions in the consumer population, development of resistant pathogenic organisms, inhibition of starter cultures, and poor consumer acceptance are the most commonly cited reasons.

A study of antimicrobial drug use in Michigan dairy herds showed that the most commonly used preparations in order of increasing frequency were the beta-lactams (penicillins and cephalosporins) tetracyclines, aminoglycosides, and sulfonamides. The researchers reported that these drugs were used in over 80% of treatments (10). The most commonly used antibiotic preparations currently licensed for use in lactating dairy cattle in Canada usually contain at least one member of the beta-lactam class of antibiotics, and this class by far has been responsible for most of the contamination found in the Ontario milk supply (1, 3, 13).

To protect the Canadian consumer, safe limits have been established on residual amounts of antibiotics in milk in the form of maximum residue levels (MRLs), also known as tolerance levels (see Table 1) (2). Dosages and milk-withholding times have been established for all licensed products to provide assurance that unsafe levels of residues will not be found in milk destined for human consumption (3).

Test methods have traditionally been based on detection of growth inhibition of a test organism and often require up to 3 h to complete (6, 12). Such testing times have not been practical for the release of tanker-truck milk at the dairy receiving dock. Therefore, much emphasis has been placed in recent years on the development of rapid-detection methods for antibiotics in milk. In the 1970s and 1980s many assay systems were developed, such as radioimmunoassays, enzyme inhibition tests, and enzyme-linked immunosorbent assay (ELISA) procedures, which offer a considerable decrease in test time (4, 5, 8, 11, 12, 14).

This study was conducted to compare the ELISA-based Delvo-X-Press test with the classical microbial inhibition Delvotest-P in regard to ease of use and detection levels of various beta-lactam antibiotics, with reference to Canada's MRLs.

MATERIALS AND METHODS

A total of 100 raw-milk samples were collected from Ontario farm bulk tanks over a 2-week period. All samples were held at 4°C and tested within 48 h. All samples screened negative for antibiotic residues using our laboratory's screening program prior to being used in the trials. The screening method involves initial testing of all samples with a microbial inhibition test, developed at our lab, that is sensitive to a broad spectrum of antibiotics and sulfonamides. Confirmation of suspicious samples is done using
RESULTS AND DISCUSSION

The standard disk assay, ELISA technology, and high-performance liquid chromatography (HPLC).

Some of the samples were spiked with different levels of beta-lactam antibiotics above and below current safe levels (penicillin G, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur) and some samples were left as negative blanks. Blind trials were conducted by two technicians at our facility using the Delvotest-P and Delvo-X-Press according to the manufacturer’s directions (9).

Briefly, the Delvotest-P is a microbial inhibition test based on the detection of growth and acid production of Bacillus stearothermophilus var. calidolactis in an agar base by the indicator dye bromcresol purple. Growth is associated with a subsequent change in the dye from purple to yellow (pH 6.8 to pH 5.2) in the absence of beta-lactam microbial inhibitors. Each kit contains test ampoules seeded with the indicator organism in a solid agar medium and separate nutrient tablets containing tryptone, glucose, and nonfat dry milk. One nutrient tablet is added to each ampoule prior to testing, followed by 100 μl of milk sample. The ampoules are incubated in a block heater for 2.5 h at 65°C and results are read according to the color change.

The Delvo-X-Press is an indirect non-competitive ELISA-based test in which a calibrated amount of (enzyme linked receptor protein) (2ml) tracer is mixed with a fixed volume of milk (2ml). The tracer will react with beta-lactams that may be present in the milk sample. This mixture is then transferred to a (B-lactam) coated tube. Only free tracer will bind to this coating. After removal of the tracer-beta-lactam complex by washing, 0.5ml color developer (enzyme substrate) is added to detect any residual tracer bound to the coating. After 4 minutes incubation 0.5ml of stop solution is added to stop the reaction. Color development in the tube is inversely proportional to the beta-lactam concentration of the sample as measured by a supplied OD reader, and a cutoff absorbance is determined by running a KH standard (B-lactam) with each test. Test results can be obtained in 8 min.

The manufacturer’s reported detection levels for both tests (9) are reported in Table 1.

RESULTS AND DISCUSSION

Both of these tests recently received approval from the Food and Drug Administration as being acceptable for use in fulfilling the testing requirements of the Pasteurized Milk Ordinance in the United States (7). As can be seen from the table, both tests performed quite well and detected most of the beta-lactam antibiotics at or below Canada’s current safe levels. The only exception was cloxacillin, which was not detected by the Delvo-X-Press in levels below 70 ng/ml in our lab. This is above the current safe level of 30 ng/ml for cloxacillin. Detection levels were slightly better in most cases on the Delvotest-P, with the exception of ceftiofur. However, this sensitivity is adequate when compared to established safe levels (1000 ppb Ceftiofur equivalent). No false-positive or -negative results were found in our particular study, which contradicts the findings of other researchers using the Delvotest-P mainly on individual cow samples (6, 15).

The Delvo-X-Press offered decreased testing times without significant loss in most test sensitivities. In addition it is fairly easy to perform, but it does require some technical ability. Each kit comes with enough equipment to perform 100 tests, and the reagents have been split into batches of three, with each batch lasting for up to 2 weeks when reconstituted. Therefore, if used continuously this test could last for at least 6 weeks. This could offer advantages for the infrequent tester such as a small dairy. However, if testing time is not a factor, the Delvotest-P should be considered because of its simplicity, low equipment requirement, and longer shelf life, which make this test more attractive to the individual dairy producer or veterinary clinic.

REFERENCES


TABLE 1. Canadian MRLs and detection levels for various beta-lactam antibiotics using the Delvotest-P and Delvo-X-Press.

<table>
<thead>
<tr>
<th>Beta-lactam</th>
<th>Delvotest-P Detection (ng/ml)</th>
<th>Delvo-X-Press Detection (ng/ml)</th>
<th>MRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GB</td>
<td>AFLSC</td>
<td>GB</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>20</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Cephapirin</td>
<td>8</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>50</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

* GB, manufacturer’s (Gist-brocades) reported detection level.
* AFLSC, detection level found at our testing facility (Agriculture and Food Laboratory Services Centre).
* No level reported in Canada’s Food and Drug Act. (Detection Level of the Most Sensitive Test).
** Ceftiofur Equivalents (Parent Drug and Metabolites).