ABSTRACT

The Delvotest P Multi plate test was evaluated by screening 100 milk samples for total antibiotic residues (penicillin G, streptomycin and neomycin). The samples were taken in conjunction with an antibiotic depletion study in milk derived from six cows treated with a multiple antibiotic, intramammary infusion product. Within the limits of sensitivity of the Delvotest, only penicillin G persisted in milk samples taken beyond 60 h, whereas in some samples, the other antibiotics appeared to be depleted as early as 48 h. More sensitive tests, however, detected neomycin (Staphylococcus epidermidis, ATCC 12228) in 50% of samples taken at 60 h and streptomycin (Bacillus subtilis, ATCC 6633) at 14.5 d after discontinuation of infusion.

Given the widespread use and need of antibiotics for treatment of mastitis in dairy cows, much effort and concern has been directed towards the proper management and monitoring of antibiotics used in such treatment in order to prevent their contamination of raw milk. Penicillin in milk may provoke allergic reactions in highly sensitive individuals (7) as well as select for resistant populations of bacteria in the general population thereby rendering antibiotic treatment ineffective. The presence of β-lactam antibiotics in raw milk presents severe technical problems in the processing of milk into cheese and cultured milk products, often requiring complete discard of inert batches.

Methods of microbiological assay for antibiotic residues have utilized the Bacillus genus of microorganisms because of their high sensitivity to the majority of antibiotics (5). In particular, use of a Bacillus stearothermophilus var. calidolactis disc assay method has found favor since its approval in 1970 by the International Dairy Federation (3), and later use in the development of a rapid cylinder-plate method in our laboratories (6). The development of PM indicating agar, containing bromcresol purple that changes color from purple to yellow as acid is produced by the growth of B. stearothermophilus var. calidolactis (3), has been refined and promoted commercially as Delvotest P (ampoule test) and Delvotest P Multi (plate test) by Gist-Brocades nv Delft, The Netherlands, for convenient, rapid, qualitative determinations of antibiotic residues in milk. Although the Delvotest P diffusion test was developed primarily for detection of >0.004 I.U. of penicillin per ml of milk, positive determinations have been made for fourteen other antibiotics of varying minimum concentrations (1).

In conducting an antibiotic depletion study on milk derived from cows treated with a multiple antibiotic, intramammary infusion product, we had an excellent opportunity to evaluate the Delvotest P. Multi test to detect not only the penicillin component but also to determine the effect of other antibiotic components from the formulation. The qualitative findings of the Delvo P Multi testings are reported and compared to results of specific and quantitative assay of the penicillin, streptomycin and neomycin components of the formulation found in the same milk samples (4).

MATERIALS AND METHODS

Reagents and apparatus

Delvotest P Multi plate test kits were obtained from GB Fermentation Industries, 2055 Bishop St., Montreal, Quebec, Canada H3G 2E8. A water bath at 64°C was used to incubate the plates.

Delvotest P Multi screening test

One-tenth ml of negative and positive control solutions and 0.1 ml of each milk sample were added to individual Delvotest cups ready prepared containing B. stearothermophilus var. calidolactis and an indicator in solid media. One nutrient tablet was added to each cup and the plates were sealed with the tape supplied. The plates were incubated in a water bath at 64°C for 3 h after which any color changes were determined. Because the medium had an initial pH of 6.6 and was purple, the growth of B. stearothermophilus resulted in the liberation...
of acid causing the indicator to turn yellow; production of a yellow antibiotic indicates a negative result, i.e., the absence of antibiotic to inhibit bacterial growth. A purple coloration indicates a positive result, i.e., the presence of antibiotic. Purple coloration throughout the whole solid medium was produced with a minimum penicillin concentration of 0.004 I.U./ml. Similarly, 6 mg of neomycin/ml and 8 mg of streptomycin/ml were the minimum corresponding concentrations of these antibiotics to produce purple color throughout the entire medium.

RESULTS AND DISCUSSION

Milk sampling was done as previously described (4). Results of the qualitative assessment of the milk samples using the Delvotest P Multi method (a) directly on each milk sample and (b) following treatment of each milk sample with penicillinase to permit differentiation between other antibiotic activity and penicillin activity are summarized in Table 1. The Delvotest method is qualitative because there is only a change in its color indicator when acid is produced by the microorganism via metabolic processes under conditions of uninhibited growth. Test samples denoted by ± indicate uncertainly in the color, often appearing as motley yellow, green and purple.

The positive response found by the Delvotest throughout the 180-h range of milk samples assessed directly (A readings) indicates that antibiotic residues were persisting well beyond the required 96-h cut-off limit (2) of the infusion product. The second (B) line of Table 1 in each instance indicates the qualitative response observed when each milk sample was first treated with penicillinase to destroy penicillin, thereby indicating any positive response was due to other antibiotic residues. Within the limits of sensitivity of the Delvotest, only penicillin persisted beyond 60 h. In some samples, other antibiotics appeared to be depleted (within the limits of the test) as early as 48 h.

To evaluate the reliability of the Delvotest as a qualitative screening test for total antibiotic content, the same milk samples were subjected to specific, quantitative assay for penicillin G, streptomycin and neomycin as described and reported previously (4).

The quantitative results of milk assay for penicillin G by both the B. stearothermophilus and Sarcina lutea cylinder-plate methods support the qualitative results of the Delvotest (4) for penicillin. The use of Delvotest to detect residues of other antibiotics in the same milk proved misleading. While streptomycin residues actually persisted for more than 14.5 d, the Delvotest indicated an absence of residues at 60 h. In conducting the streptomycin assays, parallel assays were also done on some milk samples containing penicillinase for 96 to 180 h without finding significant change in streptomycin levels (1), thereby confirming that penicillin did not interfere with the streptomycin assay. Neomycin residues in the milk of 50% of the cows were found to extend beyond 96 h (2,4).

CONCLUSION

The Delvotest, although affording a rapid, convenient and apparently reliable screening method for detecting residues of penicillin G in milk, cannot be relied upon to give a true qualitative result of total antibiotic residues in milk.

TABLE 1. Total antibiotic depletion in milk (Delvotest P Multi plate test assessment) from cows treated with a multiple antibiotic intramammary product.

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Qualitative indication of antibiotic residues at intervals in hours following last infusion:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12  24  36  48  60  72  84  96  108  120  132  144  156  168  180  348</td>
</tr>
<tr>
<td>26</td>
<td>A²  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td>190</td>
<td>B²  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td>213</td>
<td>A  +  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td>214</td>
<td>B  +  +  +  +  ±  +  +  ±  +  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td>386</td>
<td>A  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td>395</td>
<td>B  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +  +  ±  +  +  +  +  +  +  +</td>
</tr>
</tbody>
</table>

²A, Delvotest performed directly on untreated milk samples.
²B, Delvotest performed on milk samples after treatment with penicillinase.
REFERENCES


Kokubo, et al., con't. from p. 867

