THE EFFECT OF SELECTED ANTIBIOTICS UPON THE SURVIVAL OF MICROORGANISMS IN RAW AND PASTEURIZED MILKS

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Data are presented showing that 5 units of penicillin, and 10 ppm of streptomycin and aureomycin respectively, when added to milk, inhibit bacterial growth.

REVIEW OF LITERATURE

Curran and Evans studied the action of penicillin on selected spore-forming bacteria in milk. They concluded that this antibiotic had no particular application in the preservation of milk.

Similar studies by Foley and Byrne indicated that penicillin had very little value in preserving high quality milk produced and handled under good sanitary practices. Moreover, since bacterial multiplication takes place very slowly in this kind of milk, action of penicillin would not be effective against this type of bacterial growth.

EXPERIMENTAL METHODS AND MATERIALS

Antibiotics Used

Pure crystalline G. penicillin, containing 100,000 units per 60 milligrams, was used in preservation of milk. The product was obtained from Merck and Co., Inc.

Dehydro-streptomycin (2 grams) was selected as the third antibiotic in the study on preservation of milk. The compound was obtained from the Heyden Chemical Corp.

Aureomycin crystalline (500 mg) was included in this study. It was obtained from the Lederle Laboratories Division, New York City.

Procedure

Laboratory pasteurization of the raw milk was done in flasks or large test tubes. The amount of milk used depended upon the antibiotic being incorporated. The milk was heated to 145°F in a controlled water bath and the temperature held for 30 minutes.

The milk samples were held at 20°C for 72 hours. Standard plate counts were made on each milk sample before and after the different antibiotics were added in accordance with Standard Methods.

EXPERIMENTAL RESULTS

The Effect of Penicillin

One tenth ml of sterile distilled water containing 100 units of penicillin was added to 20 ml of milk. This gave a final concentration of 0.5 units of the antibiotic per milliliter of milk. Table 1 shows the results obtained.

The bacterial counts were significant in the control when compared to the milk with penicillin. Many investigators have concluded that penicillin appears to be most active when the organisms are in an active stage of multiplication. Raw milk with penicillin added showed a reduction of the microflora up to 48 hours when compared to the control samples. Moreover, the antibiotic may be responsible for bacteriostasis as shown in the initial count. When the antibiotic was added to milk and pasteurized simultaneously, it seemed to be more effective as compared to pasteurized milk and penicillin added. Pasteurized milk with no antibiotic added gave better results up to 24 hours than milk treated with

Agar Plates Showing the Effect of Penicillin on the Total Plate Count of Pasteurized Milk Incubated at 20°C for 48 hrs.

NO PENICILLIN  5 U. PENICILLIN/ML

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The Effect of Selected Antibiotics

No Streptomycin 10 U. Streptomycin/ML
Agar Plates Showing the Effect of Streptomycin on the Total Plate Count of Pasteurized Milk Incubated at 20°C for 48 hrs.

Table 1—Influence of Penicillin on the Bacterial Count in Raw and Pasteurized Milk Held for 72 Hours at 20°C. (Bacterial Counts Represent an Average of 5 Samples and are Reported as the Number of Bacteria per Milliliter)

<table>
<thead>
<tr>
<th>Storage temperature 20°C.</th>
<th>0 hr.</th>
<th>24 hr.</th>
<th>48 hr.</th>
<th>72 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no penicillin</td>
<td>12,600</td>
<td>Innum.*</td>
<td>Innum.</td>
<td>Innum.</td>
</tr>
<tr>
<td>Raw milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>penicillin 5 u./ml.</td>
<td>6,000</td>
<td>133,000</td>
<td>1,190,000</td>
<td>Innum.</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no penicillin</td>
<td>90</td>
<td>1,600</td>
<td>Innum.</td>
<td>Innum.</td>
</tr>
<tr>
<td>5 u. Penicillin/ml.</td>
<td>100</td>
<td>3,200</td>
<td>16,400</td>
<td>Innum.</td>
</tr>
<tr>
<td>Pasteurization + 5 u. penicillin/ml.</td>
<td>210</td>
<td>4,800</td>
<td>32,000</td>
<td>Innum.</td>
</tr>
</tbody>
</table>

* Too many to count

Table 2—Influence of Streptomycin on the Bacterial Count in Raw and Pasteurized Milk Held for 72 Hours at 20°C. (Bacterial Counts Represent an Average of 5 Samples and are Reported as the Number of Bacteria per Milliliter)

<table>
<thead>
<tr>
<th>Storage temperature 20°C.</th>
<th>0 hr.</th>
<th>24 hr.</th>
<th>48 hr.</th>
<th>72 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no streptomycin</td>
<td>15,100</td>
<td>Innum.*</td>
<td>Innum.</td>
<td>Innum.</td>
</tr>
<tr>
<td>Raw milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ppm streptomycin</td>
<td>14,800</td>
<td>780,000</td>
<td>Innum.</td>
<td>Innum.</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no streptomycin</td>
<td>1,150</td>
<td>24,300</td>
<td>Innum.</td>
<td>Innum.</td>
</tr>
<tr>
<td>10 ppm streptomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ pasteurization</td>
<td>101</td>
<td>1,600</td>
<td>46,000</td>
<td>Innum.</td>
</tr>
<tr>
<td>Pasteurization + 10 ppm streptomycin</td>
<td>400</td>
<td>1,350</td>
<td>29,400</td>
<td>Innum.</td>
</tr>
</tbody>
</table>

* Too many to count

The Effect of Streptomycin

Two milligrams of streptomycin, in the dry state, were added to 200 ml of milk, giving a total of 10 ppm streptomycin. The remainder of the process was carried out as described in the preceding experiments with penicillin. The results are recorded in table 2.

No appreciable reduction in the bacterial counts were noted in the raw milk with streptomycin as compared with penicillin. No doubt the effectiveness of the pasteurization process is enhanced slightly when it is carried out with milk containing penicillin.
The Effect of Aureomycin

The third antibiotic used in attempting to improve the keeping quality of raw and pasteurized milk was aureomycin. Except for a few minor changes, the procedure followed was identical with that used for the other antibiotics. Two milligrams of aureomycin were added directly to each 200 ml milk sample in order to obtain approximately 10 ppm of the antibiotic in the milk. Table 3 indicates the results obtained on the samples of milk.

When 10 ppm of aureomycin were added to raw milk, the bactericidal effect was marked after 24 hours as compared to the control raw milk.

Aureomycin had the greatest effect on the microflora of pasteurized milk. The efficiency of the pasteurization process is increased when this process is carried out on milk containing aureomycin. This illustrates the inhibiting action of aureomycin on the thermoduric organisms which usually survive pasteurization temperature.

Photographs were made of milk dilution plates to illustrate the influence of the various antibiotics studied. All photographs of the plates represent a dilution of 1 to 100 of the milk.

**Discussion**

Penicillin, streptomycin, and aureomycin showed varying degrees of inhibition of the microflora in raw and pasteurized milks under the conditions in which they were used.

It is possible to anticipate that streptomycin and aureomycin would have a greater inhibitory effect upon the total microflora of raw milk as opposed to that of penicillin. The penicillin should inhibit gram-positive organisms while having little effect upon the gram-negative types while streptomycin and aureomycin are usually effective against both gram-positive and gram-negative organisms.

The antibiotics were more effective in controlling the microflora in milk when used in combination with proper pasteurization. They tend to inhibit the organisms that are capable of surviving pasteurization temperatures.

Bacterial resistance to different antibiotics has been recognized by many investigators. This phenomenon may have an important application in the use of these compounds in the preservation of dairy products.

More studies must be made before the use of antibiotics in dairy products is recommended. Antibiotics should never be used to mask a low grade product or as a

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![Agar Plates showing the effect of Aureomycin on the Total Plate Count of Pasteurized Milk Incubated at 20°C for 48 hrs.](image-url)
PUBLIC HEALTH ASPECTS

(Continued from page 148)

farms was colder than those in milk plants.
The bacterial counts for pasteurized milk ran:

- Percentage of raw count: 2 percent
- Logarithmic average on all counts: 5,980 per ml
- Coliforms in .. less than 1° per ml
- Psychrophils: 1° per ml

The report recommends that health departments make laboratory tests of pasteurized milk at least once a week, and further states that testing should include temperature, sediment, bacterial plate count, coliform count, and the phosphatase test. Analyses for butterfat and specific gravity should be determined.

The bacterial counts for pasteurized milk were:

- Percentage of raw count: 2 percent
- Logarithmic average on all counts: 30,000 per ml
- Coliforms in .. less than 1° per ml
- Psychrophils: 1° per ml

A SANITATION PROBLEM

Continued from Page 139

problem would be so serious as to justify suitable modifications in the water supply system. A thorough cleaning of water tanks and lines may be required in some instances.

LITERATURE CITED

5. Ibid. II. Kinds. Loc. cit. 29, 238-242.

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