

WORLD HEALTH  
ORGANIZATION

REGIONAL OFFICE FOR THE  
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ORGANISATION MONDIALE  
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BUREAU RÉGIONAL DE LA  
MÉDITERRANÉE ORIENTALE

WHO/FAO SEMINAR ON FOOD HYGIENE, ZOOSES CONTROL  
AND VETERINARY PUBLIC HEALTH PRACTICE

EM/SEM.VPH/6  
1 September 1964

Lahore, 29 October - 6 November  
Teheran, 7 - 11 November 1964

ORIGINAL · ENGLISH

RESIDUES OF DISINFECTANTS AND ANTIBIOTICS  
IN MILK

by

Professor Aage Jepsen  
Food and Agriculture Organization Consultant \*

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\* Royal Veterinary and Agricultural College, Copenhagen, Denmark

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## I DISINFECTANTS

The use of chemical disinfectants in many instances has become an indispensable part of proper milk hygiene practices when thermal disinfection is not practicable. The most evident field of application is no doubt in the dairy barn where large amounts of disinfectants are used for cleaning the teats and udder of animals and for the disinfection of milking machines and other milking utensils, but to some extent disinfectants also have found a place in dairy plant sanitation.

### 1. Bacteriostatic effects' off-flavours

As long as chlorine compounds, hypochlorites and chloramines, were practically the only type of disinfectants used in the dairy industry, the problem of residues of disinfectants in milk did not attract much attention, because the marked instability of chlorine compounds in the presence of milk constituents, results in rapid decomposition with the formation of inactive ions. Experiments have shown that ordinary good disinfection practices as applied in the dairy barn may leave up to 0.25 per cent of the disinfectant solution in the milk. (P. Swartling<sup>1</sup>). With chlorine solutions (200 parts per million available chlorine) an addition to milk of more than 2 per cent is required to produce off-flavours and the bacteriostatic level (inhibition of lactic acid streptococci) is at about 10 per cent. With solutions of iodine compounds, iodophors (25 parts per million) similar findings have been reported (Danish State Experimental Dairy<sup>2</sup>).

The introduction of some new types of disinfectants about 1940 and later, especially the quaternary ammonium compounds, has, however, made the problem of residues of disinfectants in milk one which cannot be ignored. Quaternary ammonium compounds are remarkable stable in milk and retain a high residual effect. Levels of 0.00001 - 0.00005 per cent still show a bacteriostatic action in milk and hence careless use of such compounds in milk hygiene practices involve very real risks of starter failures in cheese and buttermaking. Some results from the Danish State Experimental Dairy Reports on minimum bacteriostatic levels of various disinfectants in milk are compiled in the following table.

TABLE I

	Min. bacteriostatic level for starter cultures in milk
	<u>mg per litre</u>
Calciumhypochlorite	25
Chloramine	25
Iodophor	6.25 - 12.5
Alkyl-dimethyl-benzyl-ammonium-chloride	5
Cetyl-pyridinium-chloride	0.2
Cetyl-trimethyl-ammonium bromide	2

The inhibitory action of disinfectants in milk also may interfere with results of grading the milk by means of the methylene-blue reduction test. This would however hardly result from ordinary use of disinfectants but may well occur when disinfectants have been added to the milk on purpose. On an average, concentrations must be increased tenfold above the figures listed in table I to cause a definite delay of the reduction process.

2. Laboratory methods for the detection of disinfectants and preservatives in milk.

For screening of unknown samples the use of specific chemical tests for each individual compound of course is a rather uncertain and troublesome procedure. Considerable work therefore has been invested in developing non-specific microbiological tests which would be well suited for the sorting out of suspicious samples. Recently such a method which is a modification of the Kluyver fermentation test <sup>3)</sup> has been investigated and authorized by the Nordic Committee for Food Analysis <sup>4)</sup>. The method consists of a test to show whether fermentation can be brought about in the sample under investigation by adding pressed yeast, yeast extract and glucose to the sample and adjusting the pH at 3.25. The fermentation test is carried out in a flask connected to a water-filled test-tube, in which any evolved gas is collected. The minimum levels of some preservatives in milk, detectable by this method, are listed in the following table:

TABLE 2

	<u>Threshold levels in milk by fermentation test</u>
	<u>mg per litre</u>
Sodium benzoate	200 - 250
Monobromacetic Ester	2 - 2.5
Formaldehyde	100 - 200
Alkyl-dimethyl -benzyl-ammonium-chloride	1000 - 1500

The fermentation test is not effective in detecting antibiotics because *Saccharomyces* is not inhibited by most antibiotics. Attention is drawn to the fact that proper interpretation of the applicability of the fermentation test can be achieved only when the examiner determines for himself the threshold values of the preservatives in current use under local conditions. Presumptive qualitative chemical tests which may serve as supplementary in cases where the fermentation test is not sensitive enough are Wode's test <sup>5)</sup> for chlorine compounds, the amidol test for formaldehyde <sup>6)</sup> and the Miller & Elliker test for quaternary ammonium compounds <sup>7)</sup>. Wode's test shows definite positive reaction with about 20 mg available chlorine per litre. It will react also with hydrogen peroxide. The amidol test is a reaction for aldehydes and is very sensitive. Positive results thus must be confirmed by specific chemical tests (distillate with chromotropic acid). Identification of preservatives of the aromatic acids group can be performed by means of paper chromatographic methods.

## II ANTIBIOTICS

### 1. Starter failures

Soon after the introduction of antibiotics, therapy began to suffer from technical difficulties especially in the manufacture of hard ripened cheese. These difficulties were due to the inhibitory action of antibiotics in the milk which prevented normal development of the starter cultures used in cheesemaking. Since about 1947 this problem has been extensively studied in many countries. The lactic acid

bacteria of starter cultures (*S. cremoris*, *S. diacelactis* and *Leuconostoc citrovorum*) are inhibited by various antibiotics at low levels. With the delay in acid production, the pH of milk and the fresh curd remains high and favours vigorous growth of gas-producing coliform bacteria that will spoil the cheese. Off-flavours develop, combined with faulty texture with large and irregular holes. When starter cultures are used in butter-making and the production of buttermilk and sourmilk products, similar difficulties may arise.

The inhibitory levels of some antibiotics against starter cultures in milk are approximately as listed in the following table<sup>7)</sup>.

TABLE 3

	<u>Inhibitory level for starter cultures in milk</u>	<u>Complete inhibition</u>
Penicillin	0.05 units/ml	0.1 unit/ml
Aureomycin	0.02 ug/ml	1.0 ug/ml
Terramycin	0.01 -	2.0 -
Chloromycetin	0.20 -	10 -
Streptomycin	0.04 -	10 -

## 2. Public health aspects

Later public health authorities began to object against the presence of antibiotics in milk and dairy products. Especially the observation of severe allergic reactions in humans following the administration of penicillin gave reason to suspect that penicillin in the milk supply might play some role in sensitizing the human population and also in setting off insensitized individuals the allergic shock symptoms.

Today it is the general consensus of opinion that even slight traces of antibiotics in milk and food for human consumption should not be tolerated.

3. Elimination of antibiotics in milk after intramammary treatment

Antibiotics may be excreted after intramammary treatment as well as after systemic treatment or for instance intra-uterine application. By far, the highest levels in milk are obtained when the drugs have been introduced directly into mammary gland. Parts of the dose introduced are absorbed to the bloodstream or inactivated, but the main part is eliminated with the milk on subsequent milkings. In penicillin therapy, it has been found that the concentration of the drug in the milk following treatment will decrease at an approximately exponential rate. When plotting the logarithms of the penicillin concentration an approximately straight-line regression appears within the first 10 - 12 hour period 8). For how long low level residues will persist depends upon the type of preparation and especially upon the physical qualities of the solvent base used. The level of concentration and the total amount recovered in the milk varies widely within individual cows, even when identical schemes of therapy are followed. Variations from about 8 to 80 per cent recovery have been ascertained. On an average about 50 per cent of the total dose of penicillin introduced into the mammary gland will reappear in the milk, the main part in the first two milkings following treatment 9).

4. Elimination of antibiotics in the milk after systemic or intra-uterine treatment

When large doses are used for intravenous or intramuscular injections or in intra-uterine treatment a limited excretion of the drugs may take place through the mammary gland. Milk from cows which have received 6 mill. units of penicillin intramuscularly (about 10,000 unit per kg bodyweight) may contain 0.1 - 1 unit per ml 12 hours after treatment and after 24 hours 0.01 - 0.2. In cows with low yields nearing the dry period higher concentration may be found 10).

### 5. The stability of antibiotics in milk

Antibiotics follow the water phase of the milk, and in separation of whole milk containing antibiotics, a higher level of the drug will be found in the skimmed milk, and parallel to this a lower level in the cream. This is illustrated by the following figures: A whole milk containing about 6 per cent butter-fat and 2 units/ml of penicillin was separated, and the skimmed milk contained 2.4 units/ml whereas the cream (about .50 per cent butter-fat) contained only one unit/ml <sup>9)</sup>.

The stability of penicillin is known to be about maximal at the usual pH of milk and it does not suffer any appreciable loss of activity through pasteurization. Momentary heating of the milk to 80° C likewise does not affect the biological activity of tetracyclines chloromycetin or streptomycin <sup>7)</sup>.

Antibiotics also have been found in an active state in cheese, butter and spray-dried milkpowder..

### 6. Preventive measures

To prevent the contamination of milk and milk products with antibiotics the milk of treated animals must be detained on the farm and not delivered to any dairy plant until mammary excretion of the drugs has ceased. This will include intramammary as well as systemic treatment. It is however difficult to make a general statement as to the duration of the period through which the milk will contain residues of the drugs. In the U.S.A. the labelling of antibiotic drugs for treatment by the intramammary route is required to bear a statement that milk from treated cows must not be used for human consumption for at least 72 hours after treatment, unless the manufacturer has proved that his drug will be eliminated from the milk within a shorter time. There are, however, drugs of which residues will remain as long as 6 days. The following table lists the observed duration of the period of elimination for various antibiotic drugs <sup>11)</sup>.



TABLE 4

<u>By intramammary route:</u>	<u>Residues in milk present for at least</u>
Penicillin, solutions in water	2 days
- ointment base	4 -
- special retarded	6 -
Aureomycin	6 -
Terramycin	4 -
Chloromycetin	3 -
Streptomycin	4 -
<u>By intramuscular injection:</u>	
Penicillin	1 day

In Denmark since 1954 regulations are in force to the effect that antibiotics for intramammary treatment must be administered by veterinarians only, and the owner of the treated cows is responsible for the detention of milk for treated quarters for four days after intramammary treatment. The veterinarian must instruct the farmer accordingly and inform the dairy plant manager of the treatments performed.

This system which of course will work only with a disciplined and well organized farming community, has proved very useful under our conditions. At present a revision of the existing regulations is under way which will include also systemic treatment of milking animals with antibiotics, and request manufactures of pharmaceuticals to state the duration of mammary excretion of their products, because the duration of elimination actually varies a great deal with different types of preparations. The correctness of these statements will be controlled by the public health service and it is to be expected, that the once popular retarded formulas will disappear after this.

7. Testing milk for antibiotics.

The microbiological plate assay methods are used in screening milk samples for antibiotics. For details of technical procedures reference is made to 12) and 13). It is interesting to note that certain strains of lactic acid streptococci (*s. saccharolactis*) commonly present in pooled milk produce an antibiotic substance which also may cause inhibition of starter cultures and produce false positive results in testing milk for antibiotic residues. Except for the specific inactivation of penicillin by penicillinase enzyme employed as a control there is at present no specific identity tests for antibiotics when present in milk in small concentrations. Pilegaard Andersen & Leth Jørgensen 14) reported findings of *S. saccharolactis* in infected udders as a cause of clinical mastitis. They also frequently found the streptococcus in high count pooled milk, and under favourable conditions of growth it will produce enough antibiotic to render the milk unsuitable for souring by starter cultures and produce distinct zones of inhibition on assay plates. No doubt this explains the findings reported by Jester, Wright & Welch in their 1959 survey of antibiotics in milk in the U.S.A. when they state "the antibiotic-like activity found may have been due to natural inhibitory substances that are sometimes present in milk. These inhibitory substances often increase as milk ages. In fact, fresh milk that contains no measurable antibacterial activity when first tested, may develop such activity after it has aged" 15). Further references on antibiotics produced by lactic acid bacteria are given by Anker Jul Overby 16). It seems however necessary to beware of this source of error in doing surveys of antibiotics in fluid milk. To prevent formation of the antibacterial substance from growth during incubation of assay plates with milk, it is recommended that milk should be submitted to a heat treatment prior to assay (61° for 30 minutes).

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