ANTIBIOTIC RESIDUES IN RAW MILK IN THAILAND

A. Amonsin, K. Saitanu[1] and S. Teeverapanya

Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University
Bangkok 10330, Thailand

Summary

One thousand eight hundreds and twenty two samples of raw milk were detected for antibiotic residues using *Bacillus subtilis* ATCC 6633, *B. stearothermophilus* var. *calidolactis* C 593 and *Micrococcus luteus* ATCC 9341 as test organisms, were carried out from July 1991 through June 1992. Apparent antibiotic residues were found through out the study period, except in January. The detection rate varied from 0.7% in March and May to 11% in April. One hundred and thirty six (72%) samples of the 187 screening positive samples were considered to contain only the indigenous antimicrobial agents. Of the total, 51 (2.8%) samples were positive for antibiotic residues. Among the tested organisms, *B. stearothermophilus* var. *calidolactis* was the most sensitive organism in detection of the antibiotic residues.
(**Key Words**: Antibiotic Residue, Natural Bacterial Inhibitors, Raw Milk)

Introduction

Antibiotics are used to treat clinical mastitis, metritis and other diseases during lactation period. A potential consequence of the treatment is the occurrence of antibiotic residues in milk. Milk containing antibiotics is a potential hazard to consumers, eg. allergic reaction (Borrie and Barrett, 1961; Bryan, 1951) and carcinogenic effect (Anon, 1984; Weaver, 1992). The presence of antibiotics in milk had created many problems to the dairy industry, especially the manufacture of cultured milk products (Claypool, 1984; Marth and Ellickson, 1959). To avoid these undesirable effects, many countries have developed regulatory or voluntary programme to control the veterinary drug residues in food. While there are several methods to detect the antibiotic residues in milk (IDF, 1987) bacterial growth inhibition assays are usually accepted world-wide as the screening methods of choice. The accuracy of the tests has been questioned recently (Annexstad, 1992; Cullor, 1992; Cullor et al., 1992).

Antibiotic residues in bulk milk have been documented in many countries. The violative rate varied, from 0.4 percent during 1984-85 in England and Wales (Booth and Harding, 1986) to 31 percent during 1991-92 in Iran (Guity and Navabpour, 1993). This paper reports the antibiotic residues in raw milk sampling from 167 dairy farms for a period of 12 months. The results of the antibacterial activity detected in milk, the heat-labile natural bacterial inhibitor and antibiotic residues, were also compared.

Materials and Methods

Farm milk samples

One hundred and sixty seven dairy farms located in Sara Buri Province were randomized for this study. Milk was sampled monthly during July 1991 to June 1992. Number of samples in August, September, and October, 1991 were 154, 151 and 167, respectively and 150 samples in each of the other months. In all, 1822 samples were tested. About 25 ml of raw milk were collected from the farm milk which were stored in 50 litre can and then kept in the styrofoam box containing ice for transportation. On arrival at the laboratory, they were stored at −15℃ until tested later (within 2 weeks).

Microbial inhibition assays for antibiotic residues

Bacillus subtilis ATCC 6633, *Micrococcus luteus* ATCC 9341 and *B. stearothermophilus* var. *calidolactis* C593 were used through out the study. Six assay plates, *B. subtilis* (totally 4 plates, 3 plates in Muller Hinton agar (MHA) (Difco) at pH6, 7.2 plus 0.06 (μg/ml trimethoprim and pH8, and 1 plate in minimal medium (MM)), *M.*

[1] Address reprint requests to Dr. K. Saitanu, Dept. of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.
Received March 29, 1995
Accepted July 15, 1995

luteus in MHA at pH8 (1 plate) and *B. stearothermophilus* var. *calidolactis* (1 plate) in assay medium (AM), used for detection of antibiotic residues were prepared as reported by Kondo et al., 1994.

All of raw milk samples, except samples collected in November 1991, were tested initially whithout heat treatment. Samples exhibiting inhibition clear zone on the assay plates were repeatedly tested following heating at 80℃ in a waterbath for 3 min prior to the test.

Detection of antibiotic residues was carried out as previously reported by Kondo et al., 1994. Sterile filter paper (Avantec, Toyo) with the diameter of 10 mm were used to absorb the milk samples and then placed on the six assay plates. All plates were kept at room temperature, 28℃, for 1 h allowing the residues to diffuse into the agar before incubated. The plates of *B. subtilis, M. luteus* and *B. thearothermophilus* var. *calidolactis* were incubated at 30℃, 37℃ and 62℃, respectively, for 18-24 hours. The samples were considered to be screening positive when the diameter of the inhibition clear zone appeared more than 12 mm on at least one assay plate. The samples which showed inhibition zone and subsequently lost the activity after heat treatment was considered as false positive, milk contained indigenous antimicrobial agents, and those did not lose the activity were positive for antibiotic residues.

Results

Figure 1 demonstrates the level of samples of milk exhibited the inhibition zone on at least one of the assay plates. Antibiotic residues were detected in all months except January. The highest rate of false positives were found in April accounting for 11 percent. False positive samples, milk with natural bacterial inhibitors, were detected through out the study period. In January, 3 percent were found to be screening positive but all samples were negative after heat treatment.

Table 1 demonstrates the sensitivity of the assay plates in detection of heat-labile natural bacterial inhibitors and antibiotic residues. The results indicated that 187 (10.3%) out of 1,822 tested samples were screening positive samples. There were 136 (7.5%) false positive samples, milk with natural bacterial inhibitors and 51 (2.8%) antibiotic residues positive samples. The results indicated that 72% of screening positive were false positive which indicated that the milk sample contained heat-labile natural bacterial inhibitors. In all cases *B. stearothermophilus* var. *calidolactis* showed the highest sensitivity in detection of either the natural bacterial inhibitor and antibiotic residues.

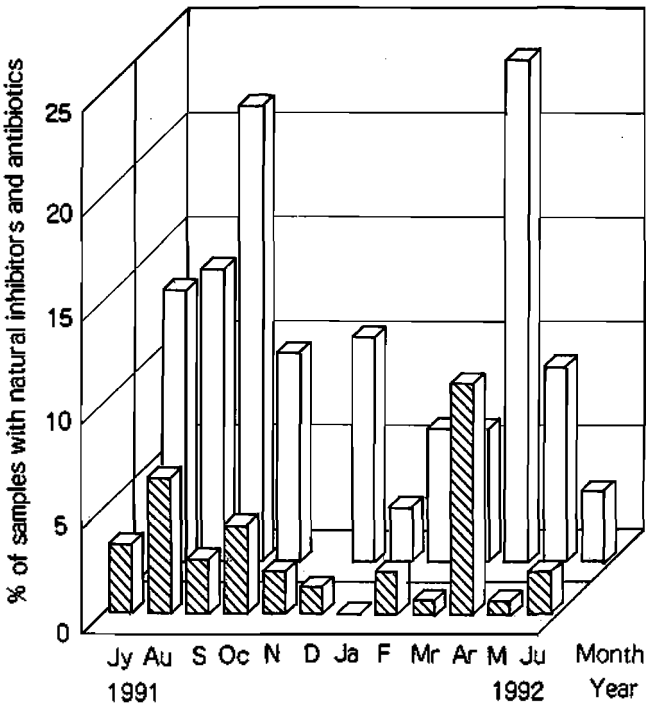

Figure. 1. Percentage of false positive, samples with natural bacterial inhibitors (□) and antibiotic residues samples (▨) of raw milk (150 to 167 per month) tested by disc diffusion assay. Samples in November were tested only after heat treatment.

Discussion

While there were several general methods for detection of inhibitors in milk (IDF, 1987), microbial inhibition tests are the most commonly used. The present study used 3 organisms namely, *B. subtilis, B. stearothermophilus* var. *calidolactis* and *M. luteus* including the media, MHA, MM and AM which enhanced the sensitivity of detection the antibiotic residues. We found that 187 samples of milk were presumptive positive but only 51 samples (2.8% of 1,822 samples) remianed positive following heat treatment which gave 72% conversion from screening positive to negative results for antibiotic residues. Even so the level of antibiotic residues (2.8%) is still considered The contaminated farm milk obviously resulting the milk in contaminated collecting tank. The 72% conversion rate was markedly high when compared with the report from U.S.A. (Oliver et al.,1990) which showed only 8% conversion rate. The data suggest that natural bacterial inhibitors, e.g. lactoferrin, lysozyme, or the lactoperoxidase thiocyanate complex, may have produced the false positive reactions (Carlsson et al., 1989; IDF, 1985; Vakil et al., 1969).

TABLE 1. THE SENSITIVITY OF 6 ASSAY PLATES ON SCREENING POSITIVE SAMPLES, SAMPLES WITH NATURAL BACTERIAL INHIBITOR AND ANTIBIOTIC RESIDUES.

Screening positive samples (187, 10.3%)*	Sample with Natural antimicrobial agent (136, 7.5%)△	Antibiotic residues samples (51, 2.8%)▽
AM, ML, MM, 6, 7.2, 8 (100%)□	AM, ML, MM, 6, 7.2, 8 (100%)	AM, ML, MM, 6, 8 (100%)
AM, ML, MM, 6, 8 (99%)	AM, ML, MM, 6, 7.2 (99%)	AM, MM, 6, 8 (98%)
AM, ML, MM, 6 (97%)	AM, ML, MM, 6 (96%)	AM, ML, MM, 6 (98%)
AM, MM, 6 (91%)	AM, MM, 6 (87%)	AM, MM, 6 (96%)
AM, MM (82%)	AM, MM (68%)	AM, MM (92%)
AM, 6 (67%)	AM, 6 (69%)	AM, 6 (80%)
AM (58%)	AM (51%)	AM (76%)
MM (28%)	MM (22%)	MM (43%)
ML (17%)	ML (16%)	ML (18%)
6 (32%)	6 (29%)	6 (37%)
7.2 (15%)	7.2 (13%)	7.2 (22%)
8 (16%)	8 (11%)	8 (27%)

*Number and percentage of samples showed inhibition zone on assay plate before heating at 80℃ for 3 min.
△ Number and percentage of samples loss antibacterial activity after heat treatmtent.
▽ Number and percentage of samples showed inhibition zone after heat treatment.
□ Percentage of the samples showed inhibition zone on the indicated assay plates.
AM, *B. stearothermophilus* var. *calidolactis* in Assay medium.
MM, *B. subtilis* in Minimal medium.
ML, *M. luteus* in Muller Hinton agar (MHA) pH8.
6, 7.2, 8, *B. subtilis* in MHA pH6, 7.2 + 0.06 μ trimethoprim and pH8, respectively.

The present data strongly indicated that the farm milk contained high levels of heat-sensitive natural microbial inhibitors. Therefore, raw milk should be preheated before test. Thirapatsakun et al. (1992) reported that 48, 40 and 47%, samples of raw milk collected from farm milk cans, storage tank at milk collection center and pasteurized milk were positive for antibiotic residues, respectively. This data, tested on non-heated raw milk samples using Delvotest-P (Gist-brocades, Netherlands), may be included a certain number of false positive as we found in this study.

In conclusion, since the present data demonstrated a high rate of false positive this indicated that raw milk contained heat sensitive natural inhibitors. We recommended that the milk should be heated before the test. The violative rate of antibiotic residues in raw cow milk was 2.8%. Validating of antibiotic residues tests should be performed before the test is recommended as the officials method in Thailand. Finally, further studies to elucidate factors responsible for the false positive must be carried out.

Literature Cited

Annexstad, J. 1992. Are residue tests accurate? Dairy Herd Management. 29, 11.

Anonymous. 1984. The hazard of using choramphenicol in food animals. J.A.V.M.A. 184:930-931.

Booth, J. M. and F. Harding. 1986. Testing for antibiotic residues in milk. Vet. Rec. 119:565-569.

Borrie, P. and J. Barrett. 1961. Dermatitis caused by penicillin in bulked milk supplies. Brit. Med. J. 2:1267.

Bryan, C. S. 1951. Problems created for the dairy industry by antibiotic mastitis treatments. J. Milk and Food Technol. 14:161-162.

Carlsson, A., L. Bjorck and K. Persson. 1989. Lactoferrin and lysozymes in milk during acute mastitis and their inhibitory effect in Delvotest-P. J. Dairy Sci. 72:3166-3175.

Claypool, I. L. 1984. Concerns and problem of cheese industry for milk quality and procurement - today and future. J. Dairy. Sci. 67:2091-2094.

Cullor, J. S. 1992. Tests for identifying antibiotic residues in milk: How well do they work? Vet. Med. 87:1235-1241.

Cullor, J. S., A. Van Eenennaam, J. Dellinger, L. Perani, W. Smith and L. Jensen. 1992. Antibiotic residue assays: Can they be used to test milk from individual cows? Vet. Med. 87:477-494.

Guity, K. and S. Navabpour. 1993. Antimicrobial residues in milk in Tehran area. In: 11th Int. Sym. WAVFH 24-29 Oct. 93. Bangkok. Ingkaninun, P. and P. Poomvises (eds.). pp. 209-211.

International Dairy Federation. 1985. Protective proteins in milk-biological significance and exploitation. Bull. Int. Dairy Fed. No. 191/1985. p. 35.

International Dairy Federation. 1987. Milk and milk products detection of inhibitor. Bull. Int. Dairy Fed. No. 220/1987. p. 78.

Kondo, F., S. Nagato, C. E. Tsai and K. Saitanu. 1994. Determination of pyridonecarboxylic acids in plasma by reverse-phase high-performance liquid chromatography. Microbiose. 77:181-189.

Marth, E. H. and B. E. Ellickson. 1959. Problems created by the presence of antibiotics in milk and milk products-A review. J. Milk Food Technol. 22:206-272.

Oliver, S. P., T. M. Lewis, M. J. Lewis, H. H. Dowlen and J. L. Maki. 1990. Persistence of antibiotic in bovine mammary secretions following intramammary infusion at cessation of milking. Prevent. Vet. Med. 9:301-311.

Thirapatsakun, T., S. Mailiang, U. Kukiatnant and R. Yuwadee. 1992. Determination for antibiotic residues in raw, pasteurized and UHT milk. J. Thai Vet. Med. Assoc. 43:21-39.

Vakil, J. R., R. C. Chandan, R. M. Parry and K. M. Shahani. 1969. Susceptibility of several microorganisms to milk lysozymes. J. Dairy Sci. 52:1192-1197.

Weaver, L. D. 1992. Antibiotics residues in milk and meat: Perceptions and realities. Vet. Med. 87:1222-1228.