

Investigation of Beta-Lactam Residues in Unpacked Milk Consumed in Sanliurfa

Mustafa ARDIÇ^{1*}

Hisamettin DURMAZ¹

¹ Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Harran University, 63300, Sanliurfa.

*e-posta: mardic@harran.edu.tr

Summary: It was aimed to investigate qualitatively the detection of beta-lactam residues in milk samples consumed in Sanliurfa region. The samples were collected in winter and summer seasons. In the experiments, *Bacillus stearothermophilus* was used as the sensitive microorganism. 96 of 300 milk samples were positive for inhibitory substances. Of positive results, 64 samples contained beta-lactam antibiotics and 32 samples were related to other residues that have antimicrobial activity. This study showed that ratio of the milk samples contained beta-lactam and other antimicrobial residue was high.

Key words: milk, beta-lactam, antimicrobial residue

Şanlıurfa'da Ambalajsız Olarak Tüketime Sunulan Sütlerde Beta-Lactam Kalıntılarının Araştırılması

Özet: Bu çalışmada Şanlıurfa bölgesinde tüketilen süt örneklerinde beta-laktam kalıntılarının kalitatif olarak belirlenmesi amaçlandı. Süt örnekleri yaz ve kış aylarında toplandı. Araştırmada antibiyotiklere duyarlı mikroorganizma olan *Bacillus stearothermophilus* kullanıldı. 300 süt örneğinin 96'sı inhibitör madde bakımından pozitif olarak belirlendi. Pozitif numunelerden 64'ünün beta-laktam antibiyotik kalıntısı, 32'sinin antimikrobiyel aktiviteye sahip diğer kalıntıları içerdiği belirlendi. Araştırma, Şanlıurfa yöresinde tüketime sunulan sütlerin önemli bir kısmının antibiyotik kalıntısı içerdiğini göstermiştir.

Anahtar Kelimeler: süt, beta-lactam, antimikrobiyal kalıntı

INTRODUCTION

Antibiotics are widely used in dairy cattle for the treatment of diseases, especially in mastitis treatment (Jones and Seymour, 1988; Ramirez et al., 2003). Of all the antibiotics, the beta-lactams (β -lactams), which include penicillin G (benzylpenicillin) are the most commonly used in veterinary medicine (Schenck and Callery, 1998; Gaudin et al., 2001).

Because of the fact that antibiotic can pass into milk, it can cause health problems resulted by allergic reaction (Seymour et al., 1988; Yamani et al., 1999; Gaudin et al., 2001). Any exposure of intestinal microflora of humans to antibiotics may lead to an increase in the numbers of antibiotic resistant species (McGrane et al., 1996; Cinquina et al., 2003). In addition, antibiotic residues cause economical losses in milk industry due to some technological problems. They may cause interference with lactic acid fermentation in the manufacture of dairy products dependent on this process (Molina et al., 2003). These residues can also influence the results of quality control tests applied to milk, such as reduction test and total bacteria count. This may lead to false interpretation of the results (Srikandakumar et al., 2004).

There are only a few studies (Temiz and Öner, 1988; Önal et al., 1993; Dokuzlu and Tayar, 2001; Koç and Akyüz, 2001) about antibiotic of milk consumed in the other regions of Turkey. In order to estimate antimicrobial

residue of milk consumed in Sanliurfa, the samples were obtained from retail markets in winter and summer seasons.

MATERIALS and METHODS

A total of 300 raw milk samples were randomly collected from Sanliurfa, Turkey, during summer and winter seasons and 150 samples were collected for each season. The samples were taken in 500 ml quantities and transported to the laboratory within an insulated container at about 4 °C for analysis.

Microorganism and maintenance of culture

The culture of *Bacillus stearothermophilus* var. *calidolactis* C 953 was supplied by Etlik Central Veterinary Control and Research Institute in Ankara, Turkey and used as test bacteria. Individual stock culture was maintained at 4 °C on triptone soya agar (TSA-Oxoid CM131) slants through bimonthly transfers.

Reagents

Penicillin G stock solution and Penicillinase disc used as confirmation were obtained from Sigma-Aldrich (Istanbul, Turkey) and BBL (BBL/Becton Dickinson and Company, USA), respectively.

Disc Diffusion Assay (*Bacillus stearothermophilus* qualitative disc method II)

B. stearothermophilus was grown in triptone soya broth (TSB- Oxoid CM129) at 64°C for 72 h. After incubation, the bacteria suspension was centrifuged for 15 min at 5000

rpm and supernatant was decanted. Then, it was resuspended and recentrifuged. The same procedure was repeated at three times. One ml of this inoculum was added to each flask containing 100 ml aliquots of the seed agar (Oxoid CM327) and poured into plates. Steril filter papers (diameter 12.7 mm) were impregnated with milk samples, which were previously pasteurized at 65 °C for 30 min. The disc impregnated with milk, penicillin (positive control) and penicillinase discs were placed on the culture medium. The plates were kept at room temperature for 30 min and incubated at 64 °C until well-defined zones of inhibition. Presence of inhibitory substance was evaluated by measuring the diameter of inhibition zone. Clear zones of >14 mm were evaluated as positive of inhibitory substances and ≤14 mm were read as negative. Then, positive samples were interpreted using penicillin G and penicillinase discs according to standard method by AOAC (2000).

RESULTS and DISCUSSION

In the present study, it was aimed to investigate qualitatively the detection of β-lactam residues in milk samples consumed in Sanliurfa. The initial screening of 300 milk samples by the Disc assay test showed that 96 of all the samples had inhibitory substances. Most (64 samples) of the positive results were found as β-lactams using the penicillinase and the penicillin G and 32 positive results were evaluated as other residues that have antimicrobial activity (Table 1). None of 204 (%68) samples were detected any antimicrobial residues.

In addition, milk samples collected during winter season were higher from β-lactam antibiotic residues than the samples collected

during summer season. Some researchers stated that antibiotic use in dairy cattle is still the most frequent reason for mastitis treatment (Meek et al., 1986; Gardner et al., 1990; Guterbock, 1995). The marked seasonal differences could be related with higher incidence of mastitis during winter months of the year. This was supported by Reneau (1993) who found that 82% of antibiotic residues were related to the treatment of mastitis.

There are conflicting between Turkish Food Codex and Turkish Foodstuffs Rules and Regulation. According to Foodstuffs Rules and Regulation, antibiotics should not be detected from the qualified milk (Olçay and Eldem, 1990). On the other hand the maximum residual limits (MRLs) of β-lactam antibiotics in milk are set by the Turkish Food Codex (Resmi Gazete, 2002).

Based on the Turkish Foodstuffs Rules and Regulation, our findings and other independent studies indicated that high ratio of the milk consumed in different regions in Turkey had an unacceptable level of antibiotic residues (Temiz and Öner, 1988; Önal et al., 1993; Dokuzlu and Tayar, 2001; Koç and Akyüz, 2001).

The literature reviews showed that there was a distinct difference in the incidence of antibiotic residues in milk between low-income and high-income countries (Table 2). It is said that antibiotic residues of milk may be influenced by several factors such as the type of analytical method and milk sample, but the difference in incidence of antibiotic residues in milk between low (including Turkey) and high-income countries is evident. And also the presence of a control system with reported low prevalence rates of antibiotic residues is likely to be the main contributing factor.

Table 1. β-lactam and other residues in milk samples

Sesion	No. of samples examined	No. of samples positive		No. of total positive
		β-lactam	Other Residues	
Winter	150	43	19	62
Summer	150	21	13	34
Total	300	64 (21.3%)	32 (10.7%)	96 (32%)

Table 2. A comparison of reported antibiotic residue prevalence in milk according to Control program in local dairies

Country	Year	Incidence	Control	References
Brazil	2000 & 2001	4.3-50%	None	Borges et al., 2000; Folly and Machado, 2001; Nascimento et al., 2001
Kenya	1999 & 2000	16%	None	Kang'ethe et al, 2005
India	1995	9%	None	Sudershan and Bhat, 1995
Poland	1995	13-22%	None	Rybinska et al., 1995
Sweden	1998 & 2003	0.26-0.08%	Present	Sternesjö and Johnsson, 1998; 2003
European countries	1990	1.2 - 1.5%	Present	Suhren et al., 1990

CONCLUSION

As a result, a lot of the milk samples in Sanliurfa region were found to have β -lactam antibiotic residues. This high incidence may be due to unconscious use of antibiotic for animal diseases and to be put on the market of milk containing antibiotic residues in Sanliurfa. These residues may cause allergic reactions in some consumers, and very likely will contribute to development of strains of bacteria that are resistant to antibiotics, thus reducing the effectiveness of antibiotic medicinals against human diseases. It is imperative that stricter quality control measures should be put into practice in Sanliurfa to assure that consumers are provided with healthy milk and milk products.

REFERENCES

- AOAC (2000). Official Methods of Analysis. 17th ed., Association of Official Agricultural Chemist, Maryland.
- Borges, GT., Santana, AP., Mesquita, AJ., Mesquita, SQP., Silva, LAF., Nunes, VQ., (2000). Residues of antibiotics in integral and standard pasteurized milk produced and commercialized in Goiás, Brazil. *Cienc. Anim. Bras.* 1, 59-63.
- Cinquina, AL., Roberti, P., Giannetti, L., Longo, F., Draisci, R., Fagiolo, A., Brizioli, NR., (2003). Determination of enrofloxacin and its metabolite ciprofloxacin in goat milk by high-performance liquid chromatography with diode-array detection optimization and validation. *J. Chromatogr. A* 987, 221-226.
- Dokuzlu, C., Tayar, M., (2001). Bursa ve çevresinde çiğ sütlerde antibiyotik varlığının belirlenmesi. *Vet. Bil. Derg.* 17 (1), 153-157.
- Folly, M., Machado, S., (2001). Antibiotic residues determination, using microbial inhibition, protein binding and immunoassays methods, in pasteurized milk commercialized in the northern region of Rio de Janeiro State, Brazil. *Cienc. Rural* 31 (1), 95-98.
- Gardner, IA., Hird, DW., Utterback, WW., Danaye-Elmi, C., Heron, BR., Christiansen, KH., Sischo, WM., (1990). Mortality, morbidity, case-fatality, and culling rates for California dairy cattle as evaluated by the National Animal Health Monitoring System, 1986-1987. *Prev. Vet. Med.* 8, 157.
- Gaudin, V., Fontaine, J., Maris, P., (2001). Screening of penicillin residues in milk by surface plasmon resonance-based biosensor assay: comparison of chemical and enzymatic sample pre-treatment. *Anal. Chim. Acta.* 436, 191-198.
- Guterbock, W.M., (1995). Rational treatment of clinical mastitis. In: Proceedings of the 2nd Western Large Herd Dairy Management Conference, Las Vegas, Nevada, USA 49-59.
- Jones, GM., Seymour, EH., (1988). Cowside antibiotic residue testing. *J. Dairy Sci.* 71, 1691-1699.
- Kang'ethe, EK., Aboje, GO., Arimi, SM., Kanja, LW., Omoro, O., McDermott, JJ., (2005). Investigation of the risk of consuming marketed milk with antimicrobial residues in Kenya. *Food Control* 16, 349-355.
- Koç, Ş., Akyüz, N. (2001). Çiğ sütlerde antibiyotik varlığının belirlenmesi üzerine bir araştırma. In: GAP II. Tarım Kongresi 24-26 Ekim. Şanlıurfa, Türkiye. 507-512.
- McGrane, P., Rowe, MT., Anger, S., (1996). Evaluation of delvotest SP and Charm AIM-96 for the detection a range of antibiotics in milk. *Milchwissenschaft* 51, 330-333.
- Meek, AH., Martin, SW., Stone, JB., McMillan, I., Britney, JB. Grieve, DG. (1986). The relationship among current management systems, production, disease and drug usage on Ontario dairy farms. *Can. J. Vet. Res.* 50, 7.
- Molina, MP., Althaus, RL., Balasch, S., Torres, A., Peris, C. Fernandez, N., (2003). Evaluation of screening test for detection of antimicrobial residues in ewe milk. *J. Dairy Sci.* 86, 1947-1952.
- Nascimento, GGF., Maestro, V., Campos, MSP., (2001). The occurrence of antibiotic residues in milk in commercial establishments in the city of Piracicaba, Sao Paulo, Brazil. *Rev. Nutr.* 14 (2), 119-124.
- Olçay, ME., Eldem, H. (1990) Gıda Maddeleri Tüzüğü. Bayrak Matbaacılık, İstanbul
- Önal, A., Aydın, N., Ayaz, Y., İşcan, D., Savaş, N., (1993). Süt ve etlerde bulunan bazı antibiyotiklerin çeşitli yöntemlerle saptanması. *Etlik Vet. Mik. Enst. Derg.* 7, 34-51.
- Ramirez, A., Gutierrez, R., Diaz, G., Gonzalez, C., Perez, N., Salvador, V., Noa, M., (2003). High-performance thin-layer chromatography-bioautography for multiple antibiotic residues in cow's milk. *J. Chromatogr. B* 784, 315-322.
- Reneau, JK., (1993). Clinical mastitis records in production medicine programs. *Comp. Cont. Ed. Pract. Vet.* 15, 497-503.
- Resmi Gazete 2002. Türk Gıda Kodeksi Hayvansal Kökenli Gıdalarda Veteriner İlaçları Maksimum Kalıntı Limitleri Tebliği. Tebliğ No. 2002/30. Resmi Gazete Sayı 24739, Başbakanlık Mevzuatı Geliştirme ve Yayın Genel Müdürlüğü, Ankara.

- Rybinska, K., Postupolski, J., Szczesna, M., (1995). Residues of antibiotics and other inhibitory substances in milk. *Rocz. Panstw. Zakl. Hig.* 46 (3), 239-241.
- Schenck, FJ., Callery, PS., (1998). Chromatographic methods of analysis of antibiotics in milk. *J. Chromatogr. A* 812, 99-109.
- Seymour, EH., Jones, GM., McGilliard, ML., (1988). Persistence of residues in milk following antibiotic treatment of dairy cattle. *J. Dairy Sci.* 71, 2292-2296.
- Srikandakumar, A., Johnson, EH., Nsanzi, H., Al-Abri, KS., (2004). Microbes and antimicrobial substances in pasteurized milk sold in Oman. *Int. J. Food Prop.* 7, 615-627.
- Sternesjö, A., Johnson, G., (1998). A novel rapid enzyme immunoassay (Fluorophos BetaScreen) for detection of β -lactam residues in ex-farm raw milk. *J. Food Prot.* 61 (7), 808-811.
- Sternesjö, A., Johnson, G., (2003). The Swedish system for detection and separation of β -lactam antibiotics contaminated milk-a practical approach. *Milchwissenschaft* 58 (1/2), 68-69.
- Sudershan, V., Bhat, R., (1995). A survey on veterinary drug use and residues in milk in Hyderabad. *Food Addit. Contam.* 12 (5), 645-650.
- Suhren, G., Hoffmeister, A., Reichmut, J., Heeschen, W., (1990). Incidence of inhibitory substances in milk for consumption from various European countries. *Milchwissenschaft* 45 (8), 485-490.
- Temiz, A., Öner, Z., (1988). Ankara'daki iki ayrı süt işletmesine gelen çiğ sütlerde antibiyotik varlığının belirlenmesi. *Gıda* 13 (4), 289-295.
- Yamani, MI., Al-Kurdi, LMA., Haddadin, MSY., Robinson, RK., (1999). A simple test for the detection of antibiotics and other chemical residues in ex-farm milk. *Food Control* 10, 35-39.