

## **Drug residues in milk**

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### **Introduction**

Drug or antibiotic residues are remnants of antibiotic drugs or their active metabolites that are present within tissues or products e.g. meat, milk and eggs from treated animals (IDF, 1995; CAC, 1998). Levels of the drug and their metabolites may persist at unacceptable levels and consumers can be exposed to them. The use of antibiotics to bring about improved performance in growth and feed efficiency, to synchronize or control of reproductive cycle and breeding performance also often lead to harmful residual effects. Concern over antibiotic residues in food of animal origin occurs in two times; one which produces potential threat to direct toxicity in human, second is whether the low levels of antibiotic exposure would result in alteration of microflora, cause disease and the possible development of resistant strains which cause failure of antibiotic therapy in clinical situations. A withdrawal period is established to safeguard human from exposure of antibiotic added food. The withdrawal time is the time required for the residue of toxicological concern to reach safe concentration as defined by tolerance. It is the interval from the time an animal is removed from medication until permitted time of slaughter. Heavy responsibility is placed on the veterinarian and livestock producer to observe the period for a withdrawal of a drug prior to slaughter to assure that illegal concentration of drug residue in meat, milk and egg do not occur. Use of food additives may improve feed efficiency 17% in beef cattle, 10% in lambs, 15% in poultry and 15% in swine. In dairy cows, the drugs are administered for treatment of mastitis through intramammary or intravenous infusions. The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage. It is evident from several studies that many animal derived foods have unacceptable high levels of drug residues. Education on prudent use of antibiotics has been observed to be particularly lacking amongst dispensers and prescribers of antibiotics. There is particularly limited information on the consequences of residues in terms of public health implications and bacterial resistance. Also, due to prevailing harsh economic conditions farmers are known to allow only a 1-day withdrawal period for milk regardless of the type of antibiotic used. In order to safeguard human health, the World Health

Organization (WHO) and the Food Agriculture Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods (FAO, 1995; CAC, 1995a). Regulatory limits for antibiotic residues have been imposed on the dairy industry in many countries (CAC, 1995a; EU, 1999; FDA, 1996; Folly & da Machado, 2001).

### **ANTIBIOTIC DRUGS**

The antibiotics used in veterinary medicines belong to 6 major groups, viz. (i) Beta-lactams (eg: penicillin), (ii) aminoglycosides (eg: gentamycin), (iii) tetracyclines (eg: oxytetracycline), (iv) macrolides (eg: erythromycin), (v) quinolones (eg: fluroquinolone), and (vi) sulphonamides eg: trimithropin). Any of the drugs belonging to these groups can appear in milk.

Sudarshan and Bhat (1995) conducted a survey on usage of veterinary drugs in Hyderabad and Secunderabad and 12 surrounding villages. They observed that tetracycline was the drug frequently used in veterinary formulations. A survey on the use of antibiotics in dairy animals by NDRI in Bangalore and surrounding areas in 2000, showed that the common drugs used for treatment in dairy animals in the area were tetracyclines, gentamycin, ampicillin, amoxycillin, cloxacillin and penicillin. The major drugs used for the treatment of mastitis in the area are beta-lactams alone or in combination streptomycin. There are only a few published data on the occurrence and levels of antibiotic residues in Indian milk samples. This could be due to (i) non-existence of regulatory laws on antibiotic residues in milk and (ii) the perception that this is not a major problem in processed milk sold by commercial dairies in our country. As milk is collected from several thousands of farmers, small quantities of contaminated milk from treated animals would be pooled with very large volume of uncontaminated milk resulting in undetectable amounts of antibiotic residues in bulk milk. Sudarshan and Bhat (1995) detected oxytetracyclin in 9% of the market milk samples collected from Hyderabad and Secunderabad; however, none of the milk samples from co-operative sector was detected for oxytetracycline.

### **IMPLICATIONS OF NON RESTRICTIVE USE OF ANTIBIOTICS IN DAIRY COWS**

#### **1. Public health aspects**

Human health problems that may result from intake of sub chronic exposure levels include allergic reactions in sensitive people, toxicity, carcinogenic effects although the validity of some of the reactions is sometimes debated. Penicillins especially, as well as other  $\beta$ -lactam antibiotics such as cephalosporins and carbapenems could cause allergies if high levels of residues persist in milk consumed by penicillin-allergic persons. Penicillin is not inactivated by pasteurization or drying and levels as low as 0.03 IU/ml has caused skin rashes. Chloramphenicol causes disruptions like aplasia of the bone marrow.

Tetracyclines residues also have the potential to stain teeth of young children. Tetracyclines can react with nitrite to produce nitrosamines which is a carcinogen.

The non-restrictive usage of antibiotics in animal rearing may lead to problems due to the presence of harmful residues in foods and raw materials of animal origin. Development and spread of antibiotic resistance represents a serious threat with potential public health implications. Dissemination of resistance traits could narrow the line of defence against bacterial infections to only a few antibiotic agents and could increase health care costs. A close relationship seems to exist between the rate of development of resistance development and the quantities of antibiotics used. There is, however, still no agreement on the significance of antibiotic use in animals and on the development and dissemination of antibiotic resistance among bacterial pathogens. Contributing to the controversy is the isolation of bacterial pathogens of animal and human origin that are increasingly resistant to most frontline antibiotics, including third-generation cephalosporins, aminoglycosides, and even fluoroquinolones. Recent studies have demonstrated that the majority of these multiple antibiotic resistant phenotypes are obtained by the acquisition of external genes that may provide resistance to an entire class of antibiotics.

The development of antibiotic resistance in bacteria is mediated by both selective pressure due to antibiotic use and the presence of resistance genes. Resistance to antibiotics is by four major mechanisms: i) alterations in the target site of the antibiotic, such as changes in penicillin binding proteins ii) drug degradation and enzymatic inactivation of the antibiotic (e.g. penicillinases), iii) changes in cell wall permeability that prevent access to antibiotics and iv) increases in the activity of efflux pumps in the cell wall which prevent accumulation of antibiotic within the cell.

There are several factors which are thought to influence the development of resistance and this include drug concentration, long-term exposure, organism type, antibiotic type and host immune status. Low-level, long-term exposure to antibiotics may in particular have a greater selective potential than short-term, full-dose therapeutic use. The judicious use of these drugs is thus of great global concern.

#### **Pathological Effects produced by Antibiotic Residues in Milk**

- Transfer of antibiotic resistant bacteria to the human.
- Immunopathological effects
- Autoimmunity
- Carcinogenicity (Sulphamethazine, Oxytetracycline, Furazolidone)
- Mutagenicity

- Nephropathy (Gentamicin)
- Hepatotoxicity
- Reproductive disorders
- Bone marrow toxicity (Chloramphenicol)
- Allergy (Penicillin)

## 2. Technological aspects

The dairy starter cultures are mainly lactic acid bacteria used in the production of a range of fermented milk products, including cheese, yoghurt, cultured butter and cultured milks. The primary role of starter cultures in cheese manufacture is the production of lactic acid from lactose at a consistent and controlled rate. The consequent decrease in pH affects a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality.

Antibiotic residues in milk are undesirable from a manufacturing perspective, as they can interfere with starter culture activity and hence disrupt the manufacture process. The concentrations of antibiotics which would cause such effects is however often higher than would be found inherent as residues in milk. Total inhibition of the starter culture has been observed to occur at approximately 60 µg/kg penicillin G. The sensitivity of starter cultures to antibiotic substances present in milk also varies considerably. Even within the same species of culture strain, differences in sensitivity are evident. Further, the response of starter cultures to residual antibiotics in milk destined for cheese or yoghurt manufacture can also be affected by the presence of other natural potential inhibitors.

## RESIDUE DETECTION METHODS

### Analytical methods

There are several methods available for screening of raw milk for the presence of antibiotic residues.

Test name	Antibiotics	Matrices	Type/Analyte principle
SNAP beta lactam test kit	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	Bulktank bovine milk	Screening test/bacterial growth inhibition
Delvotest SP	Amoxicillin, ampicillin,	bulk tank bovine	Screening test

	cephapirin, ceftiofur	milk	
Penzyme milk and penzyme III	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	Bulk tank bovine milk	Screening test/Enzymatic
Lactek cef and Lactek BM	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	bulk tank bovine milk	Screening test/Competitive enzyme system
Paralex beta lactam assay system	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	bulk tank bovine milk	Screening test/solid-phase fluorescence immune receptor
New SNAP beta lactam assay	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	raw commingled, whole bovine milk	Screening test/Antibiotic-antigen capture system
Charm II sequential and charm I cowside	Ampicillin, cephalosporin, ceftiofur, penicillin, amoxicillin	Commingled whole bovine milk	Screening test

The common tests include microbial growth inhibition assays, which involve a standard culture of a test organism seeded in an agar or liquid growth medium. Milk sample is added and the test is incubated for periods up to several hours. In the absence of inhibitory substances, the organism grows. This can be detected visually either by opacity of the agar growth medium or typically by a colour change resulting from acid production. In the presence of an antibiotic, or any other inhibitor, the organism fails to grow and a zone of inhibition or lack of a colour change is observed. Microbiological inhibitor tests are generally reliable, have high capacity and are cost-effective. They have a broad detection pattern, which on the other hand makes them unspecific. Their main disadvantage is perhaps the required typically incubation for several hours before the result can be evaluated.

Other types of methods, which can be used for routine screening of residues, include immunoassays, receptor assays and enzymatic assays. These methods can also be applied for a preliminary identification of classes of antibiotics. The majorities of these tools are quite expensive, and require instrumentation and technical skills but have the advantages of reliability, automation and fast readings of results. They are specific and typically they have poor capacity.

### **MAXIMUM RESIDUE LIMITS**

Regulatory levels have been established for drug residues in foods in the form of maximum residue limits (MRLs). MRLs for veterinary drugs refer to the maximum concentration of a residue (resulting from the use of a veterinary drug) that is acceptable in food.

### **STEPS TO PREVENT ANTIBIOTIC RESIDUES**

Dairy producers realize the importance of eliminating the possibilities of having antibiotic residues in milk and dairy beef. Producers can take the following steps to mitigate or lessen the chances of antibiotic residues.

1. Establish a valid veterinarian-client-patient relation-ship to ensure proper diagnosis and treatment of disease.
2. Implement a preventive animal health program to reduce the incidence of disease.
3. Maintain milk quality and implement an effective mastitis management program to reduce the use of antibiotics.
4. Implement employee training and awareness of proper animal drug use.
5. Only use approved over-the-counter antibiotics, according to label instructions, and approved prescription antibiotics which have the proper label.
6. Keep records of antibiotic use and identify all treated animals, including treatment protocols.
7. Use drug residue screening tests specific for the drug utilized before marketing milk and/or meat from treated animals.
8. Do not use drugs that are specifically prohibited for use in milking, dry, or growing animals.
9. Segregate and milk treated animals after, or in a separate facility from, all non-treated animals to ensure that milk is not accidentally commingled.
10. If in doubt about residue status, do not market milk and/or dairy beef from treated animals.

## **ANTHELMINTHIC DRUG RESIDUES**

Anthelmintic drugs are widely used to control roundworm, tapeworm, liver-fluke and stomach-fluke infections in food-producing animals. However, only a limited number of drugs are licensed for use in lactating animals and have a maximum residue limit (MRL) listed under European Council Regulation 470/2009/EC. There is concern that the limited number of licensed products, and the development of drug resistance, could increase the potential for off-label applications in animals. A number of these substances have undesirable toxic effects at high doses in laboratory animals, but levels detected in food are generally well below toxicity thresholds.

Anthelmintic drugs like albendazole, oxfendazole, methyl-5 (6)-phenylsulfinyl-2-benzimidazole carbamate etc showed drug residue effect in foods. Albendazole is readily absorbed from gut and rapidly transformed to various metabolites, the major metabolites being albendazole sulfoxide, albendazole sulfone and albendazole 2 amino sulfone. These metabolites can account for all residues found in milk and dairy products at any time point that are both bioavailable and of toxicological significance. Toxicological studies had shown that albendazole and its metabolites to be mutagenic. A provisional MRL for total albendazole residues of 100 ng/ml established within the European union. Makawy et al (2006) had done studies on the toxic effect of oxfendazole (MRL) on male and female mice and their fetuses. The results show that oxfendazole MRL induced a mutagenic effect in all tested cell types. Also, oxfendazole exhibit embryotoxicity including teratogenicity. The biochemical results show that oxfendazole induced a disturbance in the different biochemical contents of all tested tissues. So, we must increase the attention paid to the potential risk of oxfendazole residues in human beings and should stress the need for careful control to ensure adherence to the prescribed withdrawal time of this drug.