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UNCOVERING AND MANAGING ANTIBIOTIC RESIDUES IN MILK AND MILK PRODUCTS

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Abstract:-

Antibiotics are chemically active compounds which are used against microbial organisms either pathogenic or non-pathogenic and in microbial contamination as and when required but sometimes accidentally or intentionally antibiotics are being added to animal feeds or used for unethical treatments of milch animals or food animals making the animal derived food with antibiotic or antimicrobial residues. Therefore, it is posing high health risk in individuals consuming such animal food products. Certain antibiotic drugs and their withdrawl/ withhold period has been set with its minimum residual limit values and it should strictly followed by processors or producers, otherwise food regulatory agencies need to monitor very judiciously these issues as it is having direct impact on consumers safety. For that new detection methods have to be quickly marketed so that farmers can easily reach to these techniques. There is need of an advancement in detection techniques we have to build for proper analysis of milk and milk products for antibiotic residues. At farm level it can be reduced by implementing good management practices (GMP) like clean milk production, organic production, identification of infected animals, quarantine of carrier animals, etc. Antibiotics use as growth promoter should be strictly prohibited. Use of antibiotics should be done carefully as and when required only that to in proper dose rate and for scheduled period to avoid resistance to that antibiotic in individual. Minimum withdrawal period should be followed by the farmers after treating animals. The unintended use of and amount of drug can reduce the antibiotic residue and we need to focus on minimal use of antibiotics.

Key Words: Antibiotics, antimicrobials, treatment, residues, good manufacturing practice, consumers, withdrawl period

1. Introduction:

An increasing public health concern of antibiotic or antimicrobial resistance was caused by the overuse of antibiotics. Antibiotic or antimicrobial residues in foods like milk, meat, and egg reduces their quality and harm

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consumer health, leading to the development of antibiotic resistance in addition to other negative effects. In the present review, we have tried to explain the commonly used antibiotics in livestock sector, sources and causes of antimicrobial resistance, their impacts on the livestock products and effect of milk processing such as steralization and pasteurization on the amount of antibiotic residues in milk, along with the preventive measures and detection techniques with special attention to residues in milk.

Although the majority of antibiotic use ensues in agricultural settings, farm animal antibiotic usage has received comparatively little attention which contributes to the overall problem of antibiotic resistance and responsible for causing allergic reactions in hypersensitive individuals. Antimicrobial residues are small number of drugs or their active metabolites, which remain in the tissues or secretions/products (milk, meat and eggs) of treated animals. The frequent administration of antimicrobials to farm animals both for therapeutic use and to promote the growth may results in antimicrobial residue in their tissues, milk or eggs. These residues have important public health and economic implications for the following reasons: allergic reactions in individuals, selection of resistant pathogenic and non-pathogenic bacteria, toxicity, carcinogenicity and hindrance of certain food products. Analysis of these residues plays a key role in ensuring the food safety. The risk of residues from the milk is higher in developing countries as compared to developed ones (Kebede *et al.*, 2014). This might be related with lack of facilities for detection and regulatory bodies that control the drug residues level in foods in the form of maximum residue limits (MRLs). The MRL is defined as the maximum concentration of a residues, resulting from the registered use of an agricultural or veterinary chemical that is recommended to be legally permitted or recognized as acceptable in food, agricultural commodity, or animal feed.

2. Sources and occurrence

Mainly there are two sources of contamination:

1) Endogenous source: It includes bacterial infection or contamination with metabolic toxin produced by bacterias, which enter the animal body through contaminated food and water.

2) Exogenous source: This includes bacterial contamination, antiseptics, chemical materials and soils.

Antibiotic contamination is considered as an endogenous in nature due to its intended use for treatment of mastitis as intramammary preparations (Figure 1), intravenous infusion, for therapeutic use in diseased conditions by intramuscular or intravenous injectable route,

oral administration, and antibiotics use as feed additives for increasing growth and preventing diseases (Al-Mohana *et al*., 2010). All antimicrobial drugs administered to dairy animal enters the milk to a certain extent, and each drug is given a specific withdrawal (waiting) period, during that period concentration of drug in the tissue declines and the drug is eliminated from the animals body in the excretory ways.

The list of commonly used antibiotics in dairy animals for various purposes, its tolerance limit and withhold period is mention in **Table 1.**

Figure 1: Intra-Mammary Infusion of Drug in a Cow Affected with Mastitis

3. Various impact of presence of antibiotic residues in milk

From a public health point of view, the presence of antimicrobial residues poses various problems/potential risks for consumers (Landers *et al*., 2012).

Table 1: List of commonly used antibiotics in dairy animals for various purposes, its tolerance limit and withhold period

Name of Antibiotic	Route of Administration	Purpose	Tolerance Limit (mg/Kg)	Withholding Period (days)
Ampicillin	Systemic	Respiratory diseases	0.01	3 days
Cloxacillin	Intramammary	Dry cow mastitis	0.01	5 days
Ceftiofur	Intramuscular/subcutaneous	Metritis, foot rot, respiratory diseases	0.1	7 days
Dihydrostreptomycin Streptomycin	Intramammary	Dry cow mastitis	0.02	5 days
Chlortetracycline/Oxytetracycline/Tetracycline	Systemic/ Milk replacer	Enteritis, weight gain, pneumonia, foot rot	0.1	7 days
Neomycin	Milk replacer	Bacterial enteritis	1.5	15 days
Spectinomycin	Systemic	Pneumonia	0.2	10 days
Sulfadimethoxine & Sulfadimidine	Systemic	Shipping fever complex, bacterial pneumonia, calf diphtheria, and foot rot	0.025	14 days
Erythromycin	Intramammary	Lactating and dry cow mastitis	-	3 days (lactating cows)
Florfenicol	Systemic	Pneumonia and foot rot	-	28 days

(FSSAI regulation, 2011)

Allergies to drugs, i.e. allergic to antibiotics like penicillin or amoxicillin represent over 40% of cases. More studies have shown that the most common indication for using antibiotics in dairy cattle is mastitis, and the preferred antibiotics include beta-lactams and streptomycin. This was supported by findings from Delhi, where similar trends were observed as beta-lactam antibiotic residues were detected from 11% of milk samples (WHO, 2016). Although antibiotic residues in milk are an indicators that the animal had been provided with antibiotic drugs in the recent past.

a. Technological Impact on dairy industry

The occurrence of antibiotic residues in milk besides to an interest of consumer health, the development of antibiotic resistance has both an economical and technological impact on the dairy industry. Antimicrobial residues at or below MRL levels can influence bacterial fermentation processes involved in the production of some dairy products, such as cheese or yoghurt. Along with the inhibition of acid formation by lactic acid bacteria, they have been also reported to be responsible for inadequate ripening, off-flavours or lower flavour intensity and uneven texture in cheese. Ultimately, antimicrobial residues may lead to a deterioration of quality and monetary losses in the dairy industry by inhibiting starter cultures in dairy technological processes.

b. Health Impact on humans

A general concern linked to the widespread usage of antimicrobials at the farm level is potential development of antibiotic resistant pathogens, particularly if treatment is not diagnostically targeted. Further concern was raised that sensitive individuals may show allergic reactions to residues of the antibiotics or their metabolites, mainly β-lactam antibiotics. However, the allergenic risk is very low. Antibiotic residues in milk are of great public health concern since milk is being widely consumed by infants, youngster and adults throughout the globe. The long-term exposure to antibiotic residues in milk may result in alteration of the drug resistance of intestinal microflora. Several antibiotics are potent antigens or act as a haptens and occupational exposure on a daily basis can lead to allergic reactions.

Few commonly used antibiotics and their effect on human health:

1. Penicillin's - Hypersensitivity reactions, especially skin rashes, general pruritis (itching), difficulty in swallowing and talking, dyspnoea and urticaria, gastrointestinal disturbances.
2. Tetracycline - Bacterial resistance particularly against coliforms in the human intestine.
3. Sulphonamides - Hypersensitivity reactions, mainly cutaneous rash.

4. Chloramphenicol - Bone marrow depression which is generally dose-related, neuritis, encephalopathy with dementia and ototoxicity. A toxic syndrome in infants which is characterised by vomiting, hypothermia, cyanosis and circulatory collapse followed by death. Chloramphenicol and its metabolites could be genotoxic.
5. Quinolones - Gastrointestinal disturbances, headache, visual disturbances, insomnia, rashes, pruritus.

4. Effect of milk processing on antibiotic residues in milk and milk products

Antibiotic residues in milk and milk products can cause serious problems for dairy industry and consumers. In Indian settings, we have habits of consuming milk after thermal processing. Processing of milk help for the inactivation of antibiotics. Different processing technique like pasteurization, sterilization, boiling and drying are routinely practiced in India. Degradation of antibiotic residues after thermal treatment is depend on various factors like structure of antibiotic, type of treatment used, the matrix, the pH, and the temperature. So, it is necessary to apply control methods correctly throughout the whole milk production chain, from raw milk to all milk derivatives, in order to avoid any potential risk caused by the presence of antibiotics.

β-Lactam antibiotics like penicillin and cephalosporins, the degradation percentage was reported to be temperature-dependent. At pasteurization temperature penicillin loose its activity based on concentration manner. A sterilization procedure (120 ^{0}C for 15–20 min) induced significant decrease of β-lactams antibiotics in milk. The low stability of β-lactams under heating is reported mainly due to the high ring strain of the small β-lactone ring, which makes it susceptible to hydrolysis. Tetracyclines are mostly thermo stable as it resist the degradation. Erythromycin is also the most susceptible antibiotic to heat treatment in the macrolide family. At sterilization temperature more than 90% reduction of residues of erythromycin has observed in various studies. Almost all the aminoglycosides are heat-labile in milk. Heating at 120°C for 20 min in milk led to the reduction in residues by more than 95%. Ciprofloxacin and norfloxacin were slightly less heat-stable in milk. In various experiments on processed milk observed that the thermal degradation of sulfonamides was time-dependent.

Milk processing can help in reducing antibiotic residues in milk. Sterilization is considered as the best method to make milk safe.

5. Techniques used for detection and analysis of drug residues

The following techniques can be performed to detect the level of antibiotics residues in animal products.

1) ELISA: Enzyme linked immunosorbent assay is a immunological method based on interaction of antigen–antibody which is very specific for a particular residue. The technique consists of sandwich ELISA tests and competitive ELISA tests.
2) HPLC: High performance liquid chromatography (HPLC) allows the qualitative and quantitative detection of multi-residues in milk. Recent developments like the ultraperformance liquid chromatography systems or types of columns with improved packagings in terms of smaller size, geometry and inertness are very valuable.
3) Biosensors: Different types of biosensors have been developed in recent years as an alternative approach to screen veterinary drugs in milk. In general, these sensors usually contain an antibody as a recognition element that interacts with the analyte. The resulting biochemical signal is measured optically or converted into an electronic signal that is further processed in appropriate equipments.
4) Other : Liquid chromatography, Gas chromatography and Paper chromatography.

6. Prevention and control of antibiotic residues in milk

As the consequences of antibiotic residues being present in milk are serious, their absence must be guaranteed or if present, they have to be at safe levels for consumers. To prevent its presence, the basic approaches need to developed like highly sensitive detection tool, good farming practices and regulation policies.

a. Develop highly sensitive detection tools:

- Simple, rapid, sensitive, specific, and economic procedures should be developed to analyze the residues.
- The test should be accurate enough to avoid false negative and false positive results.
- The regular monitoring of residues concentration is very important.
- Develop simple and economic field test to identify drug residues in edible animal products.

b. Good farming practices:

- Milk from the infected animal and healthy animal should be not mixed.

- Milk of treated animal provided with antibiotic therapy should be processed separately.
- Proper biosecurity should be maintained in dairy farms to avoid infections, cross contamination.
- Proper maintainace of lactating and non-lactating animals in separate sheds/barns is required.
- Regular washing of sheds and proper grooming is essential.
- Maintaining the good hygienic managemental practices during antibiotic administration.
- Most of the antibiotics will lose activity during pasteurization, so pasteurize the milk properly.
- Rapid screening procedures for the analysis of antibiotic residues and instant grading and rejection/prohibition of milk at reception point containing antibiotics more than MRL set values.
- Irrational use of antibiotics in field veterinary practices should be avoided.
- Paying attention to proper withdrawal period/time of antibiotics for milking cows.
- Milk should be withdrawn and discarded from all of the quarters following intra-mammary infusion of antibiotics. Withdrawal period for some important antibiotics has mentioned in Table 2.

c. Regulation policy:

- Regular monitoring is the only useful approach to counteract the residue problem faced by the dairy industry in many countries.
- National surveys on residues in milk has to be performed regularly.

Table 2: Withhold period for commonly used antibiotic in dairy animals

Sr. no.	Antibiotic	Withhold period for milk (Hours)
1.	Amoxicilin	60
2.	Cloxacilin	48
3.	Ampicilin	72
4.	Ceftiofur	72
5.	Erythrocin	36
6.	Amoxycillin and Dicloxacillin	60
7.	Tetracycline	72
8.	Streptomycin	48

(FSSAI regulation, 2011)

7. Conclusions

Antibiotic residues in animal products cause great problems due to their direct effects on consumer health. It can also develop resistant bacterial flora in human. To cope up with such health hazard prevention of antibiotic residues contamination in animal products is necessary. For that new detection

methods have to be quickly marketed so that farmers can easily reach to these techniques. The regulatory authorities should concern mainly with food safety and implementation of new legislations related to animals and their consumable products. With advanced detection techniques we have to build proper analytical strategy that prevents antibiotic residues and contaminants from reaching in the food chain. Livestock owners/handlers should follow good management practices like clean milk production, identification of infected animals, quarantine of carrier animals, the use of recommended cleaning products for milking and storage equipments, proper diagnosis with only required minimal drugs should given while trearting the animal and a proper storage conditions should maintained for animals feed. Use of antibiotics as a growth promoter should be strictly prohibited. Minimum withdrawal period after antibiotic treatment must be followed by the farmers. With this the risk of the presence of residues and contaminants in milk can be greatly reduced. The proper choice of antibiotic screening test plays an important role in the effectiveness and accuracy of residue detection. The regulatory bodies should be formed and control the antimicrobial residue level in animal products before consumption.

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