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Occurrence of Antibiotic Residues in Milk: Detection and Public Health Concerns

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Abstract

There is an increase in demand for food of animal origin, due to increasing global population. Therefore, livestock owners around the globe are trying to increase animal product output which ultimately leads to enormous production pressure on the livestock. To achieve such high production goals, animals should be disease free and physiologically fit. To ensure their healthy status, antibiotics are being used extensively in therapeutic & sub-therapeutic doses to enhance production, which ultimately lead to presence of antibiotic residues in the final animal products like milk, meat, eggs etc. If such contaminated products are consumed by humans for a longer period of time, it may subsequently lead to significant health and economic issues ranging from emergence of AMR to starter failure in dairy industry. Therefore, present review focuses on various aspects of antibiotic usage in animal husbandry practices, initiative taken to combat AMR, methods used for detection of antibiotics in milk and their impact on human health.

Keywords: Antibiotic residues; Animal products; Detection methods; Human health risks.

Introduction

Animal sourced foods such as milk, meat, eggs, fish, and honey are being consumed by people across globe. They are considered nutritious and essential for growth and development of human body. But their image of being nutritious and healthy products is now getting tarnished. This is due to occurrence of pathogenic microorganism, antibiotics, pesticides, mycotoxins, hormones, heavy metals etc. in animal feed and animal sourced foods especially from India.[1-9] Among various xenobiotic, antibiotics are more commonly reported in animal sourced foods. Antibiotics are the substances which are produced naturally by living organisms or synthetically in laboratory conditions, and are able to kill or inhibit the growth of other microorganisms. Antibiotics enters the human body directly or indirectly by using antibiotics as growth promoters, feed efficiency enhancers or for treatment of various infectious diseases in animals.[10] These are one of the most commonly used veterinary medicine in relation to animal feed production and around 80% of animals used in food production are treated with antibiotics. The current global average consumption of antibiotics per kg of animal produced is >100mg/kg. Present use of antimicrobials for animal production in India is 30 kg/km2 which is expected to grow 312% by

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2030.[11]

In 1940s, the growth promoter effect of antibiotics was discovered when it was observed that animals show improved growth after feeding on dried mycelia of Streptomyces auriofaciens. Use of antibiotics as growth promoter may improve feed efficiency by 17% in cattle, 10% in lambs, 15% in poultry & 15% in swine.[12] Certain antibiotics are also used as premixes (Premixes are the mixtures of one or more active ingredients of antibiotic compounds that are exclusively used for preparation of medicated veterinary feeds). Medicated veterinary feeds are fed to animals after being mixed with animal feed. Examples of such antibiotics in medicated feed include oxytetracycline @ 4.4%, amoxicillin & ampicillin @ 0.025% owing to their high antimicrobial property and ability to resist digestion by gastric juices.

Antibiotics are administered to animals through various routes like parenteral, oral or topical. The residues level of antibiotics in animal produce are highest in the animals which are treated via injectable routes in comparison to oral routes. These antibiotic residues eventually appear in animal produce such as milk, meat and eggs. After consumption by the consumers through contaminated animal sourced foods, these residues are responsible for causing adverse health effects in humans such as carcinogenicity (oxytetracycline, furazolidone), nephropathy (gentamycin), mutagenicity, hepatotoxicity, reproductive disorders, anaphylactic shock (penicillins) and even transfer of antibiotic resistant bacteria (ARB) or antibiotic resistant gene (ARG) to humans.[12-13] In current world scenario, antimicrobial resistance has been a concerning problem and challenging towards treatment of various diseases. Apart from health effects on consumers, antibiotic residues in milk may interfere with fermentation process resulting in starter failure. This then contributes to significant economic losses in the dairy industry.

In context of above mentioned facts, the present review focuses on antibiotics usage in animal husbandry practices, their impact on public health and methods used for detection of antibiotics residues in milk.

Material and methods

The information presented in this review was obtained from a literature search of free electronic databases such as Google Scholar, PubMed, and Science Direct with regard to publications on antibiotic residues in milk. The key words used were "antibiotic", "antibiotic residues", "antibiotic residues in milk", "antibiotic residues in feed", "antimicrobial drug residues in food", "antimicrobial residues", "antimicrobial drugs in animals", "drug residues in milk", "antimicrobial drug residues in milk". The exclusion criteria included incomplete publications (i.e. papers with abstract only) and papers published in non-indexed journals. The inclusion criteria included full length original research papers, review articles, short communications in indexed and peer–reviewed journals.

Reasons for occurrence of antibiotics residues in animal sourced foods and food safety regulations in India Since there is ever increasing demand for milk & meat products, therefore antimicrobials have been an irreplaceable part of animal health care sector worldwide. For production of better quality products, the primary focus is always on the quality of raw products. Therefore, the health and disease free status of producing animal is a must from economic as well as welfare point of view. Antibiotics have been one of the most common medicine used for treatment of wide variety of infections in both humans and animals. However, residues of these drugs when excreted from body of treated animals or secreted in animal produce, may directly or indirectly enters the food chain. Direct sources of antibiotics to animals include parenteral or oral administration of antibiotic drugs, fodder mixed with antibiotics to enhance feed efficiency, intramammary or intrauterine infusion. Indirect sources include contamination of animal derived feed with water during processing, storage and transmission.[14] The contamination of feed and crops can also occur from faecal recycling as the drug residues gets excreted in the faeces of treated animals and faecal matter of animals is commonly used fertilizer in fields.[15] The major reasons for occurrence of antibiotic residues in animal sourced foods could be: (i) failure to follow a proper withdrawal period which is specific for each antibiotic drug, (ii) extra label use of antibiotics, (iii) poor record keeping of dairy animals, and (iv) mixing of milk from treated animals with the milk from untreated animals.

Consumption of such products with a higher level of antibiotic residues for a prolonged period may impart serious health risk to consumers. In order to avoid serious public health complications arising from irrational use of antibiotic drugs in producer animals, the Food Safety & Standards Authority of India (FSSAI) have established the Maximum

Residual Limits (MRLs) for antibiotic residues in various food of animal origins (Table 1).[16] Food commodities found to be exceeding these limits are not legally permitted to be sold to consumers in Indian domestic market. Similarly, other food safety agencies across the world such as European commission, US FDA, Codex Alimentarius Commission etc. have also established tolerance limits for antibiotics and other residues in milk to safeguard human health.

To tackle the issue of antibiotic misuse and AMR in India, Government of India and many NGOs have come forward with many initiatives such as an Inter-sectoral coordination committee to regulate non-therapeutic use of antibiotics in food animals has been constituted under National Policy for containment of AMR. Schedule H1 has been implemented by CDSCO and there is blanket ban on over the counter sale of almost 25 antimicrobials including cephalosporin, carbapenems, anti TB drugs & newer fluoroquinolones etc. A road map to tackle global challenge of AMR from Indian perspective was formulated in Chennai Declaration. GARP (Global Antibiotic Resistance Partnership) involving India, Kenya, South Africa, Vietnam, China are among other actions taken in this regard.

Table 1: Tolerance limit of various Antibiotic drug residues in Bovine milk.

Name of Antibiotics	Tolerance limit (mg/kg)
Monensin	0.002
Sulphadimidine	0.025
Ampicillin, Cloxacillin, Salinomycicin, Tyvalosin Tartrate, Virginiamycin, Sulphadiazine, Sulphathiazole sodium, Trimethoprim, Sulphadiazine, Sulphanilamide	
Sulphaguanidine, Bacitracin, Apramycin,Cephapirine, Clopidol, Enrofloxacin, Ethopabate, Flavomycin, Sulphaquinoxaline	0.01
Streptomycin	0.02
Colistin	0.05
Ceftiofur	0.1
Lincomycin	0.15
Oxytetracycline/Tetracycline, Spectinomycin	0.1
Neomycin	1.5

Public Health Concern of Antibiotic Residues in Food of Animal Origin

Antibiotics, if consumed for a prolonged period of time even in sub-therapeutic or low doses, may cause variety of effects on exposed population. Most common direct effects are hypersensitivity reaction (penicillin), yellowing of teeth (tetracycline), aplastic anemia (chloramphenicol) and gastro-intestinal disturbances.[17] Acute toxicity of sulphonamides leads to renal dysfunction and blood dyscrasias, while chronic toxicity leads to conditions like hepatic necrosis, hypothrombinemia. Tetracycline toxicity can also cause hepatotoxicity & nephrotoxicity. Streptomycin causes ototoxicity.[18] Certain macrolides are hepatotoxic in nature.[19] One of the major concerns is transfer of antibiotic resistance to humans which can either be by transfer of antibiotic resistant bacteria (ARB) or Antibiotic resistant gene (ARG) to humans through food chain or livestock handlers.[20] AMR is possibly the single biggest threat facing the world in the area of infectious diseases. Although little is known about magnitude of this problem with animal husbandry practices as determinant. This may be due to rare screening ofherds by the farmers, limited testing by veterinary diagnostic labs, poor surveillance mechanisms in exclusive animal pathogens compared to zoonotic pathogens, lack of resources, cost involved in culture or sensitivity testing, lack of coordination for collection, culture & antimicrobial testing methods or may be due to perceived low priority. However, this serious issue should be viewed at the same level of priorities as climate change or terrorism.

Methods for Detection of Antibiotic Residues in Milk

A plethora of analytical methods have been devised to qualitatively and quantitatively detect occurrence of antibiotic residues in food of animal origin especially milk. The various confirmatory and screening methods can be broadly grouped into four main categories viz. (i) microbiological methods based on bacterial growth inhibition, (ii) Immunochemical techniques (ELISA), (iii) Chromatographic methods (HPLC), and (iv) Biosensors based techniques.

Confirmatory tests rely on primarily on liquid chromatography coupled with diode array detector (HPLC-DAD) or with mass spectrometry (LC-MS).[21] LC-MS, a type of quantitative assay requires instrument with high response value & high sensitivity (Table 2). LC-MS method can detect multiple analytes in a single run due to its high selectivity & can detect analyte as low as ng/L or ng/gm.[22-23] Various preparation methods such as solvent-phase extraction (SPE) series like Solid-phase Microextraction (SPME) and Molecularly Imprinted Dispersive solid-phase extraction (MIDSPE) & Solvent Extraction (SE) series like Pressurized liquid extraction (PLE), Accelerated solvent extraction (ASE), and Solid–Liquid extraction (SLE) are implemented before chromatographic analysis by HPLC. Another method which is quick, easy, cheap, effective, rugged & safe (QuEChERS) has been widely used due to its low solvent consumption & high recovery.[24] It is a green chemistry based approach wherein, minimum quantity of organic solvents are utilized for extraction of targeted analytes.

Screening methods on the other hand are a type of qualitative or semiquantitative microbiological assay which are based on the reaction between susceptible organism and targeted antibiotic. Their advantages over the quantitative assay are that these are reliable, cost effective & simple to perform.[25] The other semi quantitative immunoassays are ELISA, Fluoroimmunoassay (FIA) & Time resolved Fluoroimmunoassay (TRFIA), Fluorescence-polarization immunoassay (FPIA), Immunosensors & Biosensors.[25-26] A biosensor, as defined by IUPAC, is a self contained integrated device that is capable of providing specific quantitative or semi quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element. Biosensors are classified according to the physicochemical transduction elements in electrochemical, optical, mass sensitive and thermal sensors.

Table 2: Detection of antibiotic residues in milk using HPLC.

Compounds	Sample preparation	HPLC system	Recovery	LOD (μg/L)	LOQ (μg/L)	Reference
Oxytetracycline and amoxicillin	SPE	HPLC-DAD	88-98%	1.3-2.0	3.8-6.1	27
58 Antibiotics	LLE	LC−ESI−MS/MS (+)	*	0.06−13.6	*	28
5 Fluoroquinolones	SPE	LC−ESI−MS/MS (+)	*	0.2	0.5	29
23 antibiotics	LLE - SPE	LC−ESI−MS/MS (+)	22 -143%	*	*	30
36 Antibiotics	LLE	TLX−ESI−MS/MS using optimized turbulent flow (+)	60 -105%	0.3−25.0	1.0−75.0	31
6 β-lactam antibiotics	LLE	LC−ESI−MS/MS (+)	42 - 81%	0.4−10.0	1.0−25.0	32
5 Fluoroquinolones						
and 1 quinolone	LLE	LC−DAD−ESI− MS (+)	64 - 96%	2.4−30	8−110	33
21 antibiotics	LLE	LC−ESI−MS/MS (+)	67 - 128%	0.1−2.5	0.1−5	34
33 Antibiotics	SPE	LC−ESI−MS/MS (+) and (−)	*	0.01−3.70	*	35
38 Antibiotics	LLE - SPE	LC−ESI−MS/MS (+)	68- 118%	0.01−5	0.03−10	36
6 Antibiotics	QuEChERS	LC−ESI−MS/MS (+)	84 -116%	0.4−3	0.1−0.5	37
25 Antibiotics	modified LLE method	LC−ESI−MS/MS (+)	62 -108%	0.2−10	2.5−25	38
6 Cephalosporins	MISPE	LC−ESI−MS/MS (+)	15 - 100%			
± 6%	0.1−3.8	0.4−12.5	39			
61 Antibiotics	LLE-SPE	LC−ESI−MS/MS (+)	62- 119%			
± 10%	0.003−1.57	0.001−5.18	40			
16 Macrolides and metabolites						
	QuEChERS	LC−ESI−MS/MS (+)	62 - 115%	0.30−0.85	1.1−4.0	41
15 Antibiotics	SPE	CZE−Q-TOF−MS	76 - 106%			
± 13%	0.5−2.9	1.5−9.6	42			
20 Antibiotics	dSPE	LC−ESI−MS/MS (+)	70 - 97%			
± 10%	0.10−2.40	0.33−7.92	43			
4 Antibiotics	LLE	LC−ESI−MS/MS (+)	61 - 111%	0.1−0.5	1.0−5.0	44

* Not available

Conclusion

Since antibiotics play a major role in keeping the livestock sector free from diseases, their occurrence in the foods of animal origin is also a major public health hazard & one of the alarming situations due to rise in number on antimicrobial resistant microorganisms, which often becomes a challenging task to treat. Veterinarians can play a major role in controlling the emergence of antibiotic resistant bacteria by judiciously using antibiotics with broad spectrum action and shorter withdrawal time. Livestock owners should be made aware about the consumption of produce from animal which is under treatment or have been recently treated with antimicrobials. Since, it may not be practically feasible to nullify the use of antibiotics in animals as the pathogens are omnipresent but precautions should always be taken while administering such drugs to animals. The ban on over the counter sale of antibiotics to avoid irrational use by livestock owners, restricting use of drugs in sub-therapeutic doses, avoid mixing of antibiotics in feed as growth promoters are some of the strategies which can probably minimize the risks associated with antibiotic residues in foreseeable future. In advance clinical settings, it is advisable to initiate antibiotic treatment only after the laboratory report of antimicrobial sensitivity testing. Action plans need to be devised in order to control such conditions. We cannot control the evolutionary development of microorganisms which are turning resistant to antibiotics on their own but the least we can do is to avoid the indiscriminate use of antibiotics which is an enabling factor for them to develop resistance against these magic bullets. Newer and old revisited approaches can move us from the end of antibiotic era towards a new dawn of antibacterial agents.

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