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Antimicrobial Residues in Cow Milk and its Public Health Significance

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Abstract: Antimicrobial residues are small amounts of drugs or their active metabolites which remain in the tissues or products such as meat, milk and eggs from treated animals. Almost all drugs administered intentionally or unintentionally to animals result in trace residues remains in food products. Inflammation of mammary gland has implications for the areas of the dairy industry. The frequent administration of antimicrobials to farm animals both therapeutically and to promote growth may result in antimicrobial residue in tissues, milk or eggs. These residues have important public health and economic implications for the following reasons: allergic reactions, selection of resistant pathogenic and non-pathogenic bacteria, toxicity, carcinogenicity and hindrance of certain food products. The most important cause for occurrence of antimicrobial residue in animal tissues is insufficient period of time given for the drug to be eliminated from food and must be considered a practical impossibility. Therefore, the maximum residue limits (MRL) set for each antimicrobial agent should be respected. Conducting appropriate screening tests to determine residue status can aid in maintaining a safe milk supply. For prevention and control of antimicrobial residues, veterinarians and producers should stick to the prescribed withdrawal times of antimicrobial agents and test presence of residues when necessary.

Key words: Antimicrobials · Drug residues · Milk

INTRODUCTION

Antimicrobials are used in dairy cattle production primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. The use of antimicrobial therapy to treat and prevent udder infections in cow is a key component of mastitis control in many countries. Due to the widespread use of antimicrobials for treatment of mastitis in dairy cow much effort and concern has been directed towards the proper management and monitoring of antimicrobials used in such treatment in order to prevent contamination of raw milk. However, wide spread use of antimicrobial has created potential residue problems in dairy products [1].

Antimicrobial residues are small amount of drugs or their active metabolites, which remain in the tissues or products (meat, milk and eggs) from treated animals. Problems associated with antimicrobial residues in milk include the risk of allergic reactions after consumption by penicillin sensitized persons and increased resistance of pathogenic bacteria towards antimicrobials. The concerns arise mainly from the possibility that antimicrobial resistant bacteria may be transferred from livestock to humans, through animal to human contact, through environment or in contaminated food products [2].

Levels of the drug and their metabolites may persist at unacceptable levels and consumers can be exposed to them. In dairy cows, the drugs are administered for treatment of mastitis mainly through intramammary infusion. The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra- label use of drugs and incorrect dosage. Prolonged drug excretion, or unauthorized drug use may result in residues of these substances in milk and tissues [3].

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Different kinds of methods were developed to detect antimicrobial residues. The first step consists commonly in a wide screening to detect as many antimicrobials as possible. These methods are based on the various susceptibilities of bacteria to different antimicrobials. The most common screening methods for antimicrobial drug residues are based on the growth inhibition of a test microorganism [4].

To protect the consumer, safe limits have been established on residual amounts of antibiotics in milk in the form of maximum residue level (MRL). Milk withholding times have degree of assurance that milk kept for human consumption will not contain quantities of antimicrobial residue that might be harmful to humans [5].

In order to safeguard human health, the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods [6]. Regulatory limits for antimicrobial residues have been imposed on the dairy industry in many countries [7]. However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk. Based on the above facts, the objectives of this review paper were to provide an overview on the antimicrobial residue in milk and to discuss the public health hazards and economic impact of antimicrobial residues in milk.

Antimicrobials in Dairy Production

Commonly Used Antimicrobials in Dairy Cows: There are currently a number of antimicrobials approved for intramammary use in lactating cows (Table 1). Each of these antimicrobials has a prescribed withdrawal period. After a cow has been milked a full lactation, she is dried off to prepare her for calving in the next lactation. Although they are no longer milking, dairy cows are still susceptible to mastitis. For this reason, nearly all cows are treated with long acting intramammary antimicrobials [8].

Antimicrobial Administration and Residue: Administration of antimicrobials to dairy cattle is usually therapeutics that is in response to development of symptoms of disease. These types of chemotherapy shorten the period of antimicrobial administration and usually reduce the amount of antimicrobials employed. The use of feed and water grade antimicrobial is prohibited in milking cows, so most antimicrobials are administered orally or given by infusion or injection.

Table 1: Examples of common antimicrobial agents administered to dairy cattle

Antibiotics Family	Examples
Amino glycosides	Gentamycin
Cephalosporin	Cephaprin
Ionophores	Monensin
Macrolids	Erythromycin, Tylosin
Penicillin	Ampicillin, Penicillin and Cloaxacillun
Tetracycline	Oxytetracycline

Source: Moore *et al.* [8]

Intra mammary treatments are subject to two mechanisms; biological (distribution in udder tissue and transfer in to blood) and physical (mechanical elimination of the milk at each milking). Several antibiotics with very high diffusion rates will rapidly pass through the different membrane (plasma membrane and vessel walls), entering the blood circulation and being excreted in different ways particularly in urea. However some molecules will stay in the teat and udder. When a milking dairy cow is treated with an antimicrobial, the cow's milk must be withheld for certain period. The producer must discard this milk and receives no payment for it. All loads of milk are tested for antimicrobial residues to ensure that milk containing residue does not inadvertently enter the food supply [9].

Reasons for Antimicrobial Residues in Milk: The most likely cause of violative drug residues is the failure to observe withdrawal times [10]. Improper maintenance of treatment records or failure to identify treated animals adequately may lead to their omission. Violative drug residues can also occur as a result of improper use of licensed product through the illegal use of unlicensed substances. Extra-label dosages and use of drugs which have not been approved for the species in question may lead to violative residues [11]. The disease status of an animal and the way in which drugs are administered influence the potential for residues. Disease may affect the pharmacokinetics of the drug, metabolism or the presence of infection and /or inflammation may cause the drug to accumulate in affected tissues [11]. The significance of contamination depends on the pharmacodynamics of the compound and the species affected [12].

The main reasons for the occurrence of antimicrobial contamination in milk is from a treated cow is accidentally routed in to the pipeline, an antibiotic treated dairy cow is unintentionally milked, the same milking unit is used to milk an antibiotic treated cow before milking untreated cows, the milking unit is not cleaned and sanitized between uses, lactating cows are purchased and the new

owner is unaware of recent antibiotics treatment prior to sale, one quarter of a cow is treated for mastitis and withheld from the bulk tank, equipment used to milk treated cows is handled carelessly and all antimicrobial treated dairy cows are milked last, but the milk line was not diverted from the bulk tank [13].

Antimicrobial residues occur when employees fail to follow the specific label instructions when treating cows. They may also occur when treated cows are accidentally milked into the bulk tank before withdrawal period is completed. Residues may also occur when employees fail to clearly identify treated cows with chalk marks, leg bands or neck chains. They may also occur when written records for treatment are not kept or are not checked prior to returning the treated cow to the milking herd. Treated cows should be housed and milked separately from milking herd [13].

Significance of Antimicrobial Residues in Milk

Public Health Significance: The non-restrictive usage of antimicrobials in animals rearing may lead to problems due to the presence of residues in food and raw materials of animal origin. Human health can either be affected through residues of drugs in foods of animal origin, which may cause direct side effects, or indirectly through selection of antimicrobial resistance determinant that may spread human pathogen [14]. Human health problems that may result from intake of sub chronic exposure levels include allergic reactions in sensitive people, toxicity and carcinogenic effect. Penicillin especially, as well as other â- lactam antibiotics such as cephalosporin could cause allergies if high level of residues persists in milk consumed by penicillin allergic persons. Tetracycline residue also has the potential to stain teeth of young children [15].

Antimicrobial Resistance: The use of antimicrobials in food animals can result in antimicrobial resistant bacteria reaching the human population through variety of routes. Antimicrobial resistant bacteria such as *E. coli* can colonize the intestine of people. Healthily exposed humans (farmers who use food containing antimicrobials, slaughter house workers, cookers and other food handlers) often have incidence of resistant *E. coli* in their feces than general population [16]. While many bacteria are not pathogenic, some bacterial species from intestine of animal cause zoonotic infection to human such as *Salmonella* species, *Campylobacter* species. Development and spread of antimicrobial resistance represents a serious threat with potential public health implications [16].

Hypersensitivity Reaction: It is an immune mediated response to a drug agent in a sensitized patient and drug allergy is restricted to reaction mediated by IgE. Drugs are foreign molecules, but their molecular weight is usually too small to be immunogenic, they act as haptens, which must combine with drug sensitive person to be immunogenic and elicit antibody formation [17]. Allergic reactions to antimicrobials may include anaphylaxis, serum sickness, cutaneous reaction and delayed hypersensitivity reactions. These effects are acquired after human beings consume food of animal origin, which contain drug residue that has allergic effects of the antimicrobials employed as food additives or in chemotherapy penicillin and streptomycin appear from clinical use in humans to be more included to produce hypersensitivity or allergensity than others in present use. About 50% of the human population is considered to be hypersensitive to a number of substances including penicillin [18].

Carcinogenic Effect: Carcinogenic effects refer to an effect produced by a drug having carcinogenic or cancer producing activity. Among the carcinogenic veterinary drugs in current use in many countries are nitrofurans, nitromidazoles and quinoxaline. These drugs are acquired via food of animal origin as antimicrobial residues. The potential hazards of carcinogenic residue are related to their interaction or covalent binding with various intracellular compounds such as proteins, ribonucleic acid, glycogen, phospholipids and glutathione. This leads to change cellular components such as DNA [18].

Mutagenic Effects: The term mutagen is used to describe chemical agents that damage the genetic component of a cell or organism. Genetic material of all living organisms, with the exception of some virus is DNA. Several chemicals including alkylating agents and analogs of DNA bases have been shown to elicit mutagenic activities. There has been an increasing concern that drugs as well as environmental chemicals may pose a potential hazard to the human population by production of genes mutations or chromosome aberrations. Either the general or somatic cell may be affected understandably; injury to either cell group may lead to serious consequences. However, from public health, mutation in the general cells is more immediate importance because of the hazard to further generation [18].

Teratogenic Effect: The term teratogen applies to a drug or chemical agent that produces a toxic effect on the embryo or foetus during a critical phase of gestation.

As a consequence, a congenital malformation that affects structural and functional integrity of organism is produced [18].

Environmental Impact: Animals may excrete metabolites of antimicrobials through urine and feces and reach the soil and water. The most prevalent antimicrobials found in the environment (surfaces of water) belong to the macrolid and sulfonamide groups. Tetracycline or penicillin has only been found in some cases and at low concentrations [19]. Some commonly used antimicrobials such as erythromycin, sulfadimdine and tetracycline are antimicrobials which persist in the soil and remain on the surface of water and soil for a period of over year [20].

Antimicrobial metabolites have also been found to be transformed back to their origin active substances once in the environment. Since most antimicrobials are water soluble, up to 90% of a dose can be excreted in urine and up to 75% in animal feces [21].

Dairy Industry Impact: The dairy industry is an important segment in the food industry, providing both milk and meat for human consumption. Dairy farmers and all supporting groups (veterinarians, feed supply dealers, milk processors, livestock dealers etc.) should be concerned and devoted in producing as safe as well nutritious dairy food products. The dairy starter cultures currently used in dairy industries for the primary acidification of milk belong mainly to the genera Lactococcus, Streptococcus and Lactobacillus. These starter cultures are mainly lactic acid bacteria used in the production of a range of fermented milk products, including cheese, yoghurt and cultured butter. The primary role of starter culture 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 [22]. Antimicrobial residues in milk are undesirable from a manufacturing perspective as they can interfere with starter culture activity and hence disrupt the manufacture process [23].

Safety Evaluations: Regulatory levels have been established for drug residues in food in the form of maximum residue limit (MRL). To assess the safety of ingested antimicrobial residues national and international committee evaluated data on chemical, pharmacological, toxicological and other properties [24].

Acceptable Daily Intake (ADI): Acceptable daily intake (ADI) for a given compound is the amount of a substance that can be ingested daily over a life time without appreciable health risk. Calculation of ADI is based on array of toxicological safety evaluation that takes in to account acute and long term exposure to the drug and its potential impact. This defines a maximum quantity which may be consumed daily by even the most sensitive group in the population without any outward effects. The ADI is determined as a consecutive estimate of a safe ingestion level by the human population based on the lowest no effect level (NOEL) of toxicological safety studies [25].

Maximum Residue Level (MRL): The maximum residue level (MRL) is the maximum concentration of residue resulting from the use of a veterinary medicinal product that may be legally permitted or recognized as acceptable in or on a food, allocated to individual food commodities. The MRLs are fixed on the basis of relevant toxicological data [26]. Substances for which no maximum residue limit can be established because residues of these substances, at whatever limits in food stuffs of animal origin constitute a hazard to health of the consumer [27]. Maximum residue level of some veterinary drugs in milk is shown in Table 2.

Withdrawal Period: Use of animal medicines requires observance of the withdrawal period. This is the time between the last doses given to the animal and the time when the level of residues in the tissues (muscle, liver, kidney, skin and fat) and products (milk, eggs, honey) is lower than equal to the MRL. Until the withdrawal period has elapsed, the animal or its products must not be used for human consumption [28].

Antimicrobial Residue Detection Methods

Screening Tests: Screening tests are used to detect the presence of an analyte or a class of analyte at the level of interest. They are aimed at avoiding false negative results while false positive results are tolerable. These tests when used for substances with an established maximum residual limit, the detection should be as low as possible [30].

The most common screening methods for antimicrobial drug residues are anti-micro biological tests, based on the growth inhibition of microorganism (e.g. *Bacillus stearothermophilus*). There are on farm screening tests devised for rapid detection of low concentrations of antimicrobial residues in milk [31]. The most common assay system monitors inhibition of

Table 2: Residue limits of some common veterinary drugs (µg/kg) set for milk

Antimicrobials	MRL(µg/kg)
Procain benzyl penicillin	4
Streptomycin	200
Diaminazine	150
Oxefendazole	100
Neomycin	100
Oxytetracycline	500
Sulfadimidine	25

Source: CAC [2]

Table 3: Discard time for milk of some antibiotics in dairy cows:

Drug	Discard time for milk prior to sale
Ampicillin	48 hours
Erythromycin	72 hours
Procaine benzyl penicillin	72 hours
Sulphadimethoxine	60 hours

Source: Prescott and Baggot [29]

the growth of a test organism. This type of assay system cannot identify the nature of the compound responsible for the growth inhibition. A well-known assay in this category includes charm inhibition assay and delvotest. The assay systems of some of the newer residue detection tests are based on immunobinding of unique antigenic structures in antimicrobial or inherent antimicrobial receptor interaction [32].

Charm inhibition assay uses *B. stearothermophlus* in tablet form and a specially formulated agar medium. The antimicrobial substances in the milk sample inhibit microbial sporulation and growth, which results in reduced acid production. The pH indicator changes from blue to greenish yellow if the milk does not contain an inhibitory substance, it remains blue if the milk contains a growth inhibitor [4].

Delvotest is an agar diffusion test that utilizes *B. stearothermophlus.* It is promoted as an on-farm method of detecting antimicrobial residues. In this test, the content of the test organism ampule and the nutrient tablet are mixed. The milk sample is combined with the mixed content. The mixture is incubated for 2.5-3 hours at 64°C and the color reaction is recorded based on a pH change in the media. A yellow color indicates a negative reaction and purple color indicates a positive reaction [4].

Confirmatory Tests: These methods provide full or complementary information enabling the analyte to be identified unequivocally at the level of interest. The tests are employed to determine presence or absence of residues in a sample found positive by routine screening test. These tests are aimed at preventing false positive results [33].

Commonly used procedures for detection of veterinary drug residues include high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC) and mass spectrometry (MS) [34]. Chemical methods usually proceed with a preliminary extraction in order to isolate drug of interest from biological matrix. The main objectives of sample treatment are removal of macromolecules and other matrix constituents that may either adversely affect the chromatographic systems or interfere with the detection and enrichment of the analyte in order to achieve the required low limits of detection. The low solubility of some antimicrobials in organic solvents has made it difficult to develop procedures to extract and concentrate their residues from biological matrices. Liquid chromatography has merged as the method of choice for determination of antimicrobial which are rather polar, non-volatile and sometimes heat stable [34].

Prevention of Antimicrobial Residues: A food control system is an official institution setup, at national and sub national level, responsible for ensuring the quality and safety of food supply. It include the relevant food legislation and regulation, food inspection, food analysis, food import /export inspector and certification and food control management. It is important to remember that the individuals must likely to come in to first contact with antimicrobial resistant bacteria in the dairy are the dairy producers and their families [35].

Providing on farm food safety programmes, which address the daily management of the production until with regard to animal health and well-being, public health and environmental health must be a top priority for agriculturalists and veterinarians. Developing critical control point management (CCPM) products for animal and human health concerns is viable approach to aid in alleviating public concern about dairy products and the food supply in general. The safety and quality of dairy products will improve as people realize that health animals are more profitable, which encourages them to pay attention to diagnosis and treatment of disease, prevention, costs less than cure and images quality can increase the attractiveness of milk on market [36]. Milk quality assurance program will become a valuable tool in maintaining a safe and wholesome product [13].

The following are some of the prevention strategies of drug residues: practice healthy herd management; establish a valid veterinarian patient relationship; use prescription drugs with a veterinarian's guidance and maintain milk quality and food safety begins from the dairy products; implement an effective mastitis

management program; administer drugs properly and identify treated animals; use drug residue screening test; maintenance and use of proper treatment records in all treated animals; implement employee/family awareness of proper drug use to avoid marketing adulterated milk and complete the milk and residue prevention protocol annually.

CONCLUSIONS

Dairy management has a role in the complex tasks of producing high quality milk management and employee must become very knowledgeable about the milk quality factor and tasks that impact each factor training of producer and other holder is a key point to improve quality. Any control strategy should be implemented alongside educational measure to the producer on antimicrobial use and adoption of sound management practices. The correct identification of the causative agent of the disease and strict adherence to antimicrobial label recommendations is one of the easiest ways of reducing the likelihood that antimicrobial resistant bacteria will enter the food chain. In conclusion, milk producers should be aware about risks with antimicrobial residue as a result of failure to respect the withdrawal time.

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