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Antibiotic Residues in Cow Milk and its Public Health Importance Review

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Abstract: Antibiotic residue in primary dairy product (milk) is unimportant amounts of drugsor chemicals, which are active metabolites and remain in milk after treating lactating cows. The most important reason of antibiotic residues in the milk is animal treatment with antibiotics. The frequent administration of antimicrobials to dairy animals both therapeutically, metaprophylaxis and or 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 obstruction 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 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: Antibiotic · Residues · Milk · Withdrawal Period

INTRODUCTION

Antibiotics are vital drugs considered as the ultimate strategy to treat animal infections. Their effectiveness is however, threatened by extensive and inappropriate use of these, not only in cattle but also in human medicine. Antibiotics have been used in the dairy industry for more than five decades in dairy cattle production to treat or prevent disease and to increases milk production or improve feed efficiency [1].

In veterinary practice, antibiotics are utilized at therapeutic levels primarily to treat diseases and to prevent infections [2]. They are also used at sub-therapeutic levels to increase feed efficiency, promote growth and prevent diseases [3]. The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products of animal origin, such as milk or meat. Presence of drugs or antibiotics residues in food above the maximum level recognized world-wide by various public authorities is illegal [2]. The most commonly used antimicrobials in food producing animals are the â -lactams, tetracyclines, aminoglycosides, lincosamides, macrolides and sulfonamides [4]. In situation where proper withdrawal period is not observed or not precisely known by cattle keepers [5] drug residues in milk is likely to occur.

Residues of drugs in milk and meat is a potential health risks because of failure to observe the mandatory withdrawal time/periods, illegal or extra-label use of veterinary drugs and incorrect dosage levels application [6]. Effects of antibiotic residues include selection of antibiotic-resistant bacteria which could later be transferred from animals to humans, through contaminated milk products [7, 8]. In addition to residual effect of drugs to human consumers, antibiotic residues in milk affect industrial processing of milk to other products. A fermentation step is frequently employed for the manufacture of food products, such as cheese and yoghurt, from milk. Milk contaminated with antibiotic residues and subsequently employed in the

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manufacture of cheese or yoghurt, can result in the inhibition of culture development, due to elimination or reduction of the micro-organism necessary to allow fermentation [9, 6].

Consequently as a result, milk and milk products contaminated with antibiotics beyond a recommended residue levels, are considered unfit for human consumption [10]. Good quality milk has normal chemical composition, completely free from harmful bacteria and harmful toxic substances, free from sediments and extraneous substances, which have lower degree of titratable acidity, of good flavor, adequate in preserving quality and low in bacterial counts [11]. High-quality milk apart from containing low bacterial count also contains a low number of somatic cells and is free of human pathogens and antibiotic residues [12]. Testing for microbiological quality, chemical and antibiotic residues are not practiced at small scale dairy or milk collection centers in developing country and the test done for raw milk wholesomeness at the collection centers is only lactometer test of specific gravity [13].

To protect the public against possible health risks caused by drug residues hazards, regulations regarding veterinary use of drugs including withholding periods after antibiotics therapy and tolerance levels have been formulated by FAO/ WHO -CAC [14] and are strictly adhered in developed countries [4]. However, such regulations are not usually adhered in developing countries where routine monitoring of veterinary drug residues in food is not done [15]. Milk contamination can be reduced through effective good hygienic practices from farm level to the final consumer [16]. Maximum Residue Limits (MRLs) are the levels of drug legally permitted and recognized as acceptable in a food, resulting from the correct use of a veterinary drug, which should occur in food. Maximum residue limits are based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the acceptable daily intake and an additional safety factor. MRLs give an indication of food safety and provide standards [14].

Maximum residue limits concentration of a residue, resulting from the registered use of an agricultural or veterinary chemical that is recommended to be legally permitted or recognized as acceptable in or on a food, agricultural commodity, or animal feed. The concentration is expressed in mg/kg of the commodity or mg/L in the case of a liquid commodity or ppm/ppb [17]. Regulatory limits for antimicrobial residues have been imposed on the dairy industry in many countries [16].

However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk. In spite of the aforementioned prevailing situation and the presence of a number of public health problems due to antibiotic residue resulting from the consumption of different food items in Ethiopia, there is lack of well-documented review on antibiotic residue in milk and public health importance or significance. Therefore, the objective of this paper is to review sources of antimicrobial residues in milk, public health impacts and understanding on ways of control and prevention of antibiotic residues.

Definition of Antibiotics: Abraham and Newton [18] defined the antibiotics as "antibiotic is a natural compound produced mainly by microorganisms or are compounds obtained by chemical or microbiological modification of natural compounds. Nawaz [19], also defined antibiotic as miracle drugs that are extensively used for the treatment and prevention of infectious diseases in animals and humans. They concluded that antibiotics have greatly enhanced humans and animal's life, reduced mortality and improved quality of production.

Antibiotic Residues: Are small amounts of drugs or their active metabolites, which remain in the tissues or products (meat, milk and eggs) from treated animals [20]. Antibiotic residues in milk: are small amounts of veterinary drugs or their active metabolites which remain in milk after treating various animal diseases like mastitis [21]. Inappropriate use of these antibiotics by small scale livestock keepers at farm level may lead to various bacterial pathogens developing resistance to most commonly used antibiotics which in turn increases bacterial resistant to almost all existing antibiotics [8].

The consumption of a product with this kind of residue may have a reflection on reactions such as dermatitis, asthma and rhinitis, not to mention the fact carcinogenic effect that some pharmaceutical products, including nitrofuranes and chloramphenicol have in laboratory animals, thus representing a potential risk. The fact is that even pasteurization does not eliminate the residue of antibiotics [22]. Presence of antibiotic residues in milk may be the result of failure to observe the mandatory withdrawal periods, incorrect dosage levels and/or illegal or extra-label use of drugs [7].

Source of Antibiotics Residue in Milk: The major source of antibiotic residues in the milk of animal is treatment with antibiotics. Antibiotic substances can be excreted

even after treatment, which is the reason in many countries for time limits to when the milk is not to be used for human consumption. This time may vary according to national laws and recommendations of the manufacturer [23]. Other potential ways antibiotic residues could end up excreted in commonly milk is if the cow would consume it through the feed or the drinking water [7, 8].

The extended and excessive usage of approved drugs or prolonged drug clearance and multiple dosing as the sources of antibiotics in milk [24]. Other sources include poor identification of cow under treatment and poor communication between milkers; poor record of treatment and failure to observe the recommended label withdrawal time or lack of advice on withdrawal period. On the other hand Shitandi and Sternesjö, [9] reported that the small-scale producers, with fewer resources and lacking of relevant knowledge, may not follow recommended treatment regimes when using antibiotics, as manifested in the higher prevalence of antimicrobial drug residues in their milk [25, 26].

Antibiotics Used in Dairy Cattle: Antibiotic are found in different groups which are available for treatment of infected livestock. The most common groups include the beta-lactams, sulphonamides, aminoglycosides, macrolides, tetracycline's and chloramphenicol [27-29]. These antibiotics may be used singly or at times in combination when treating dairy cattle. In addition, the presence of antibiotic residues in milk be used in dairy industry can have adverse effects on production of fermented dairy products such as yoghurt and cheese [30, 28].

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. 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 urine. 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 [31].

Problems Associated with Milk Containing Antibiotic Residue

Public Health Significance: About 90% of antimicrobial use in most of developing countries is delivered to clients without prescription [32]. This led to misuse of antimicrobials in animals that may cause antibiotic residues in animal products which have impact in human health. The health hazards attributed to antibiotic residues in animal products includes; toxic effects, transfer of antibiotic resistant bacteria to humans, carcinogenicity (e.g: sulphamethazine and oxytetracycline), mutagenicity, nephropathy (e.g: gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g: chloramphenicol) and allergy (e.g: penicillin) [33, 34]. Studies indicated that, suboptimal doses of Tetracyline promote antimicrobial resistance, allergic reaction in humans, risk of teratogenicity when administered in the first trimester of pregnancy and permanent discoloration of teeth in infants or children of less than 12 years old consuming contaminated milk whereas little has been done to control its applications [35-38].

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 [39]. 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 [39].

Carcinogenic Effect: Carcinogenic effect refers 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 nitromidazoles and quinoxaline [40]. 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 in cellular components such as DNA [41].

Teratogenic Effect: The term teratogen applies to a drug agent that produces a toxic effect on the embryo or fetus during a critical phase of gestation [40]. Consequently, a congenital malformation that affects the structural and functional integrity of the organism is produced. The well-known thalidomide incident involving a number of children in to hazard that may occur when such agent is administered during pregnancy [42].

Hypersensitivity Reaction: Allergic reactions to antimicrobials may include anaphylaxis, serum sickness, coetaneous 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 allergicity than others in present use [43]. About 50% of the human population is considered to be hypersensitive to a number of substances including penicillin [44].

Environmental Impact: Animals may excrete metabolites of antimicrobials through urine and feces and reach the soil and water. Some commonly used antimicrobials such as erythromycin, sulfadimidine and tetracycline are antimicrobials which persist in the soil and remain on the surface of water and soil for a period of over year [45]. 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% can be excreted in urine and up to 75% in animal feces [46].

Economic Effects: Antibiotics residue in milk are known to interfere with the manufacture of these dairy products through inadequate curdling of milk and partial or total inhibition of starter culture growth in making fermented dairy products [47, 48]. The products require use of starter cultures which are living bacteria and the presence of antibiotics in the milk will negatively affect flavor of the product. Milk that contains antibiotic residues causes problems of acidification and ripening of cheese which leads to loss to the processor. The residues cause the production of poor products that were discarded and hence were a loss to the processor. Also drug residues inhibit starter cultures used in production of cheese, fermented or cultured milk and other dairy products [6, 7, 9, 49, 50]. Heat treatment of milk such as boiling and pasteurization destroys or eliminate pathogenic microorganisms but have limited or variable effects on drug residues [9, 6].

Risk Assessment of Residues: International organization such as Codex Alimentarius Commission have taken initiation of harmonization of antibiotic residues in food through establishment of statutory limitations, maximum residue limits, acceptable daily intake levels, acute reference dose, no observed adverse effect levels etc.

Definition of Maximum Residue Limit (MRL): MRL is defined as the maximum concentration of a residue, resulting from the registered use of an agricultural or veterinary chemical that is recommended to be legally permitted or recognized as acceptable in or on a food, agricultural commodity, or animal feed. The concentration is expressed in milligrams per kilogram of the commodity (or milligrams per liter in the case of a liquid commodity) or ppm/ppb [17]. Regulatory levels have been established for drug residues in foods in the form of MRLs [51].

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 of toxicological safety studies [52].

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 or equal to the MRL. Depending on the drug product, dosage form and route of administration, the withdrawal time may vary from a few hours to several days or weeks. It is the interval necessary between the last administration to the animals of the drug under normal condition of used and the time when treated animal can before the production of safe foodstuffs [53]. Until the withdrawal period has elapsed, the animal or its products must not be used for human consumption [54].

Detection of Antimicrobial Residues in Milk: For the assurance of food quality, detection methods for the antimicrobial residues in milk are commonly used worldwide. The methods are categorized in two major groups such as; screening and confirmatory assays. Examples of screening assays are Enzyme Linked Immunosorbent Assay (ELISA), biosensor and microbial inhibition tests whereas confirmatory assays are high performance liquid chromatography (HPLC) [55-57].

Qualitative Assays Screening: The antimicrobial residue screening methods that are readily available use different methods and test microorganisms. The commonly used qualitative assays are microbiological, enzymatic and immunological assays [55, 56, 58].

Microbial Growth Inhibition Assays: The assays employ the use of the standard culture of the test microorganism in liquid or solid medium. Milk sample to be analyzed is applied on the agar surface and the plates are incubated for diffusion of the sample into the medium and if the sample contains inhibitor agents, inhibition of growth occurs of the tested microorganism [54]. The positive test is indicated either by formation of a clear zone of inhibition around the disc or a change in the colour of medium. 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* [56].

The advantages of these assays is that they have a wide detection spectrum, simple to carry out, reliable and they are cheap and can be used for the screening of a large number of samples. Microbial inhibitor tests detect a wider range of antimicrobial substances, including β -lactam antibiotics and give a result within 3 hours or less [37, 55, 56, 58].

Receptor/Protein Binding Assays: The principle of these assays is a protein conjugating to an enzyme. The conjugate binds to the free â -lactam antibiotics that may be present in a milk sample. The assays are used for the detection of â -lactam antimicrobial residues in the bulk milk or milk from individual lactating animals. Antibiotic specific receptor proteins or penicillin-binding proteins (PBP) are incorporated in commercially available tests [56, 58, 59].

Immunoassays: The immunoassays work on the principle of specific antibody -antigen reactions. Immunoassays are well known for their high sensitivity and specificity, simplicity and are less costly. They are useful in quantitative/semi quantitative assays of antimicrobial residues in milk. They can be used for screening antimicrobial residues in milk and preliminary identification of classes of antibiotics [37, 58 , 60].

Confirmatory (Quantitative) Assays: These are physico-chemical methods based on chromatographic principles of analysis. They are involved in isolation, separation, confirmation and quantification of specific analytical antimicrobial residues contained in milk. They have high sensitivity and specificity, high precision and capable of quantifying specific analytical test at low concentrations. The chromatographic methods used in antimicrobial residue quantification include High Performance Liquid Chromatography (HPLC), gas chromatography, ionic chromatography, size exclusion chromatography, superficial fluid chromatography and affinity chromatography [57, 61].

Status of Milk Residues in Ethiopia: In Ethiopia, the control of drugs from the government authorities and information on the actual rational drug use pertaining to veterinary drug use is very limited. In addition, misuses of drugs are common among the various sectors including veterinary and public health. In addition there is lack of awareness and preparedness among the controlling authorities and producers in dealing with the risk of indiscriminate use of antibiotics to the livestock and to the consumers [62].

No formal control mechanisms exist to protect the consumer against the consumption of meat and milk product containing harmful drug residues in the country [63]. In Ethiopia, few studies have indicated the existence of antibiotic residue in milk and other animal products. In Central Ethiopia the prevalence of oxytetracycline and penicillin, G Residues in milk and meat showed that higher than maximum residue limits [64].

Prevention and Control of Antimicrobial Residues in Milk: The responsibility for residue control and prevention cannot lie solely within a governmental agency; rather the responsibility must be shared by the government, producers, veterinarians, teachers and academicians, marketing associations and other interested parties, who must strive for both healthy and efficiently grown animals as well as a safe food supply. Several approaches can be taken to achieve this goal [65]. The control of antimicrobial residues in animal derived foods has been adapted from Darwish *et al*. [66] and

IDF Fact sheet [67]. The risk of antimicrobial residues in foods of animal origin can be controlled by abiding to two main pillars namely:

- Avoiding antimicrobial residues in animal foods by adequate management practices and a careful use of antimicrobials. It can be adhered by observing preventive measures to keep animals healthy and strictly prescribing withholding times in case of treatments.
- Effective screening with suitable analytical methods and procedures to detect eventual contaminated batches throughout the food chain as early as possible and discarding. The following are the control tips expounding the information contained in the above pillars:

The effective prevention of infectious diseases and the adoption of strict hygiene standards, reduction of unnecessary prophylactic treatment in animals, strict national legislation must be passed around the world to avoid the unnecessary use of antibiotics; national surveillance and monitoring of antimicrobial residues in foods; updating of the MRLs in animal foods, antimicrobial use in feed additives should be abandoned; avoid the use of antibiotics for treatment of animals without a veterinarian's prescription; avoidance of antimicrobials lacking clearly documented pharmacokinetics and pharmacodynamics properties, effective mastitis management program, proper administration of drugs and identification of treated animals, use of drug residue screening test maintenance and use of proper treatment records in all treated animals. Milk quality assurance program will become a valuable tool in maintaining a safe and wholesome product [68].

CONCLUSION

Antibiotics are usually used for the prevention and treatment of animal diseases and to improve the efficacy of animal production, however antimicrobial residues are remnants or small amounts of antimicrobial drugs or their active metabolites which remain in milk after treating lactating cows. These residues in milk are often due to farmers failing to adhere to the specified milk withdrawal periods after antibiotic use to sick lactating cows, illegal or extra label use of drugs and incorrect dosage levels and route of administration. 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 inhibition of starter cultures used in production of different milk products. For this reason to protect the consumer, safe limits have been established on residual amounts of antibiotics in milk in the form of maximum residue level. In order to safeguard human health, the WHO and FAO have set standards for acceptable daily intake and maximum residue limits in foods. However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk. Therefore, based on the above conclusions the following recommendations are forwarded.

- Avoid using antibiotics in the veterinary field without veterinarian's prescription.
- There should be reduction of unnecessary prophylactic treatment in animals.
- Reducing antimicrobial residues in animal foods by adequate management practices and careful use of antimicrobials.
- Until the withdrawal period has elapsed, the animal or its products must not be used for human consumption.
- Milk producers should be aware about risks with antimicrobial residue as a result of failure to respect the withdrawal time.
- Study should be conducted in Ethiopia to determine the level of antibiotic in milk and improving of the MRLs in other animal food.

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