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Global Efforts to Reduce Antibiotic Residue in Milk and Milk Products: Current Status and Future Directions – A Review

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A B S T R A C T

Antibiotic residues in milk are crucial public health, economic and technological issue. This review collates evidences from PubMed, Clarivate, Scopus, and Google Scholar, references were organized, summarized, and analyzed. Antibiotic residues sources are primarily due to therapeutic use of dairy cows and are most prevalent after intramammary treatments with beta-lactams, tetracyclines, and sulfonamides. Or as result of poor recordkeeping, misdiagnosis, or accidental contamination. Public health hazards include allergies, antimicrobial resistance, gut dysbiosis and potential carcinogenicity. From an economic point of view, antibiotic residues in dairy products cause disruption of dairy processing (i.e., fermentation failures), lead to trade sanctions and erode consumer trust. Maximum residue level (MRLs) differs depending on the country of use, making it difficult to comply and trade products on a global scale. Detection is based on both rapid screening (microbial inhibition assays, immunoassays) and confirmatory techniques (LC-MS/MS), This methodes recommended with the support of biosensors and nanotechnology may enhance the detection senstivity, the obsticles remain as results of lake of global MRLs and availability of resources, to reduce the impact of antibiotic residue the studies consensus that better farming and strict antibiotic managment with cooperation of one health context can solve this critical issue. to ensure milk safety, and industrial sustainability, and prevent AMR issue aggravation.

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Introduction

The milk is cosidered a main nutrient source for humans and animals, providing them with all essential elements required for health, growth and physiological body function , like vitamins, minerals, proteins, and carbohydrates [1]. recently, the demand for dairy products, including milk and milk-based products, has significantly increased as result of rising consumption rate [2]. Antibiotic are wildly use to protect the health of animal and human because of ability to inhibition of growth of bacteria and protozoa there are present about 250 type of anibiotic that can use for treatment [3].

Ruminants are highly susceptible to infections because of their grazing in environments that are frequently contaminated with pathogens. In modern dairy farms, antibiotics are commonly used to treat many clinical conditions and maintain herd health, However, this extensive use of antibiotics can lead to contamination of milk during production and processing. furthermore, the increased using of antibiotics in the treatment of cattle and sheep poses potential risks to human health, including the development of antibiotic resistance and other adverse effects [4].

The extensive use of antibiotics in livestock precipitate in the excretion of antibiotic residues into milk and other animal products [4]. The consumption of such residues can promote antibiotic resistance toward pathogenic microorganism. Continuous exposure to low doses of antibiotic residues through milk and dairy products lead to development of resistant bacterial strains [2]. nowadays, antibiotic resistance is responsible for approximately 700,000 deaths annually, the following study pretend that ratio will rise to 10 million by 2050. Moreover, antibiotic resistance is linked to an increased risk of diseases such as, cardiovascular disease, diabetes and obesity [5].

The antibiotics in food that originate from animals' act in two ways on the human body: either as a toxin to humans or through exposure to low levels of antibiotics, which can disrupt the normal flora of the gastrointestinal tract (GIT) [3]. This review aims to explore global efforts to reduce antibiotic residues in milk and milk products, examining the current status and proposing future directions as its primary focus.

Sources of Antibiotic Residues in Milk

The main route which antibiotic residues pass to the milk supply is though therapeutic or prophylactic treatment of dairy cows, especially through intramammary infections. Other by systemic infections, preventive treatment during the dry period and with contaminated feed or water. Beta-lactams like penicillin, amoxicillin, cephalosporins, tetracyclines, sulfonamides, and aminoglycosides are among the most frequently used classes of antibiotics in dairy farming, these antibiotics often the detected as residues in milk and milk products [6,7].

The failure of application the prescribed withdrawal periods "the time required after the antibiotic administration" is the most common reason for deposition of residues. Factors contributing to withdrawal period violations include inaccurate records monitoring, extra label drug use without appropriate withdrawal time calculation, incorrected diagnosis of treated animals, and accidental milking of treated cows into the bulk tank (Table 1) [8,9].

Public Health Importance

The concerns of consuming milk with antibiotic residues included:

• Allergic reactions: consumers with hypersensitive to specific antibiotics, like penicillin. Ingestion of even small amounts can initiate allergic reactions which ranged from mild rashes to severe anaphylaxis. While the threshold concentrations are challenged, the potential risk required strict control [10,11].

• Antimicrobial resistance (AMR): This is the most long-term outcome of continuous exposure to sub-therapeutic levels of antibiotics through contaminated food, can apply specific pressure on bacteria within the human gut, promoting the proliferation of resistant bacterial strains. These resistant bacteria can cause infections that are difficult to treat. also, resistance genes can be transferred horizontally between different bacterial species, intensify this issue. The relation between antibiotic use in livestock and AMR in humans is the foundation of the "One Health" approach, explaining the interconnectedness of human, animal, and environmental health [12,11].

• Disruption of gut flora: Antibiotic residues can disrupt the balance of the human intestinal flora, this might lead to dysbiosis, which associated with various health issues, including gastrointestinal disorders and suppress immune function [13].

• Carcinogenicity/Toxicity: less clearly known for most used veterinary antibiotics at residue levels, concerns about long-term toxic effects, including carcinogenicity or mutagenicity for certain compounds, contribute to the need for stringent controls [6].

Economic and technological importance

Antibiotic residues create an economic and technical issue in dairy industry which include :

Milk Refusing : if the residue exceed the MRLs limits , many dairy prcessing centers and dairy industry refuse it as a product leading to massive economic losses , specially those contaminated with beta-lactams which destroying lactic acid bacteria and prevent fermentation in yogurt, cheese and other

products, leading to change in the texture and flavor [8,7].
Trade Barriers: dairy companies in many countries have a strict rules to accept antibiotic residue contaminated products, result in shiping rejection and harmful international trade relationships [14].
Responding to Consumer Confidence: Public awareness of food safety issues, including antibiotic residues, is rising. Perpetrated incidents diminish consumer confidence in dairy foods and the industry [14].

Maximum Residue Limits (MRLs)
Maximum Residue Levels (MRLs) represent the highest legally permissible concentration of residues of veterinary drugs (including antibiotics) in foodstuffs of animal origin, including milk. These limits are set according to stringent toxicological evaluations that are designed to prevent undesirable consumer exposure beyond the Acceptable Daily Intake (ADI), the level of a contaminant that can be eaten daily over a lifetime without causing appreciable risk or harm. These bodies, including institutions such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), carry out the risk assessment, and the Codex Alimentarius Commission generally set MRL standards at the international level, which then act as references for international trade [15,11].

MRLs can differ markedly between countries or regions (e.g., European Union, United States, Canada, Japan, Australia). For example, the EU, MRLs are usually adopted as agreed recommendations from the European Medicines Agency (EMA) to be applied on the same way at all member states [16,17]. Under the Pasteurized Milk Ordinance (PMO), the US Food and Drug Administration (FDA) establishes "tolerances" which are equivalent to MRLs for veterinary drug residues in milk (Table 2) [14].

Regulatory and Monitoring Challenges
The challenges facing dairy industry regardless continuous residues processing, resulting from different national policies in risk evaluation. The challenge lies in areas that lack accurate resources or have weak governantal control. Furthurmore , the monitoring programs must continue to cope to emerging veterinary drugs control and sophsticated analysis technology [14,18].

Methods of Antibiotic Residue Detection
A wide range of screening or confirmatory tests are available to investigate antibiotic residues in milk products including:
• Screening Tests
They are usually low-cost, rapid tests designed to process many samples quickly, screening out potentially non-compliant ones. They are commonly applied at farm, collection point, or at reception at dairies [19].
• Microbial inhibition assays
These are traditional tests (e.g., Delvotest®, Copan Milk Test®) based on the general principle that the presence of antibiotic residues in a screened milk sample will inhibit the growth of a susceptible test bacterium (e.g., Bacillus stearothermophilus or Geobacillus stearothermophilus). Inhibition is typically indicated by a color change in pH indicator dye. They are broad-spectrum and able to detect multiple classes of antibiotics (particularly beta-lactams and sulfonamides), but not very specific and may give false positives/negatives in some cases. Their sensitivity may not always be as low as the MRLs established for some compounds [20].
• Receptor-Binding Assays (Immunoassays)
These tests (SNAP®, Charm®, ELISA kits) utilize specific antibodies or receptor proteins that will bind to antibiotic molecules. These formats include lateral flow and enzyme-linked immunosorbent assays (ELISAs). These tests tend to be quicker, more specific to a narrower range of organisms and, for certain drug classes (e.g., some beta-lactams and tetracyclines), are more sensitive than microbial inhibition tests. Most rapid kits are intended for on-farm or truck-side applications, which can help make rapid decisions about whether or not to accept milk. Multi-residue immunoassays are becoming available which can simultaneously detect different classes of antibiotics [21].

Confirmatory Methods
Specimens that screen positive must be confirmed by precise laboratory-based methods with high sensitivity. These methods providing clear identification and quantification of the certain residue [22].
• Chromatographic Methods with Mass Spectrometry no single chromatographic method is available to detect all classes of antibiotic; however, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is defined as the gold standard for confirmatory analysis. LC will separate the different components in the milk extract, which are then characterized by MS/MS: A very sensitive detection method that provides high specificity for detection, which allows unambiguous identification and quantification of the antibiotic molecules in very low (ppb or ppt) concentrations. Other methods for diagnosis include high-performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescence detection (FLD), which are less sensitive and specific than LC-MS/MS. These methods are high throughput but relatively expensive, necessitating specialized equipment and expertise, restricting their applications for routine screening [23].
• Emerging Technologies

Further research continues to develop new detection methods that are faster, cheaper, more sensitive, portable, and capable of multi-residue analysis [24].

• Biosensors

These are devices that combine a biological recognition element (such as an antibody, enzyme, aptamer, or whole cell) with a physical transducer (electrochemical, optical, or piezoelectric) for the detection of target analytes. They had the potential for rapid, sensitive, and on-site detection of antibiotic residue. Antibiotic detection in the milk has gained popularity with Surface Plasmon Resonance (SPR) and electrochemical biosensors [24].

• Nanotechnology

Nanomaterials (e.g., gold nanoparticles, quantum dots, carbon nanotubes) are being integrated into detection platforms for enhanced sensitivity and simplified assay formats, whilst commonly being used alongside immunoassays or biosensors [25].

• Spectroscopic methods

Spectroscopic techniques such as Raman spectroscopy and Near-Infrared (NIR) spectroscopy have been evaluated for use in rapid, non-destructive screening of milk, but challenges do exist in terms of obtaining the sensitivity and specificity needed for low-level residues [26].

Different situations require different solutions, e.g., rapid screening at intake points versus regulatory confirmation in accredited laboratories. Developing affordable and user-friendly screening methods remains the focus of pro-active multi-residue screening, which will also be suited for on-farm or resource-limited settings [14].

Current Strategies for Residue Reduction

The best strategy is to prevent antibiotic residues from entering the milk supply through a multifaceted approach targeting responsible antibiotic use and farm management practices [27,7].

Good Agricultural Practices (GAP) and Good Veterinary Practices (GVP)

• Judicious Use of Antibiotics

Antibiotics should be reserved for clinically diagnosed conditions, selected based on the narrowest effective spectrum, and administered at appropriate doses for the minimal required duration. Prophylactic use should be avoided unless absolutely necessary [27,7].

• Adherence to Withdrawal Periods

Critical practices include identifying treated animals (e.g., leg bands), maintaining detailed treatment records (drug, date, dosage, withdrawal period), and discarding milk during the withdrawal period [28,29].

• Veterinarian-Client-Patient Relationship (VCPR)

Collaborative decision-making between farmers and veterinarians ensures proper diagnosis, treatment protocols, and withdrawal times, especially for extra-label drug use [7].

Prevention of Cross-Contamination

Secure storage and proper handling of veterinary products prevent accidental contamination of feed or milking equipment [27].

• Biosecurity and Herd Health Management:

Reducing disease prevelence like mastitis by vaccination, nutrition, and decrease of antibiotic use [30,31].

• Farmer education on residue risks

By providing a records for antibiotics withdrawal periods, and programs or workshops for farmers and animal owners to explain health , environmental and economic impacts of antibiotic residue [29].

Alternative Therapies and Prevention Strategies

Vaccination: Wide range of vaccines are avalaible to immunize animals against mastitis different pathogens such as Staphylococcus aureus, E. Coli etc. [28].

Genetic Selection: Breeding cows form selective breed should be raised specially those known for their resistance against mastitis [7].

Herbal therapy and Essential oils: these oils have good therputical effect for treating effected teats but still under scientifc investigating [28].

Probiotics and Bacteriocins: Research explores competitive exclusion of pathogens [29].

Bacteriophages: studies showed that viruses targeting mastitis causing bacteria are effecient as an alternative preventive method [32].

Immunomodulators: this including all the substances that enhances the response of immune system is promising [31].

Enhanced diagnosis procedures: rapid test should be used to evluate unnecessary use of antibiotic [28].

Current Status and Global Prevalence

Antibiotic residues in milk vary geographically:

Developed Countries (EU, North America, Australia, NZ), these counteries applicated a strict instructions regard the hurmful residues that should be kept under (<1%) [33,27].

Low- and Middle-Income Countries (LMICs): Higher residue rates recorded as result of weak regulations, limited veterinary services, farmer unawareness, and economic pressures [29,34].

Future Directions and Research Needs

Enhancing global prevention: international efforts should applicated as a preventive regulation specially in LMICs [35].

Developing of detection technologies : a new tools should be developed s to enable the detection and monitoring of agricultural products, water, and soil. screening tools that work beyond beta-lactams detection should be improved [36].

Better farm management and technology: adopting accurate farming technologies like automated sensors to detect early treat cows accordingly, and better manage withdrawal periods [37].
Advancing the One Health Agenda: Tackling antibiotic residues and AMR necessitates a coordinated response across human, animal, and environmental health sectors. Ideally, surveillance should connect antibiotic use data, data on residues in the food chain and AMR patterns in pathogens of both humans and animals. [38].
Consumer Awareness and Demand Educating consumers about low-residue milk can create market demand for such products that are certified under strict quality assurance schemes, thereby providing economic incentives for better practices throughout the supply chain [36].
Data analytics and predictive modelling: Exploiting large datasets obtained from monitoring programs, farm records and AMR surveillance may be used to help target high-risk areas and/or practices and derive predictive models for the occurrence and distribution of residues that can be used to inform more focused interventions [39].

Conclusion

The antibiotic residues in milk remain the global buzzing alarm in the health of human and animal , and the global concern due to its environmental and economic impact, countries around the world decrease the maximum residue limits and tighten regulation and polices, this enhance monitoring programs, farming practices and develop detection technologies. The high residue levels in low- and middle-income countries remain the crisis required international efforts. The sustainable solutions to reduce antibiotic residue can be acheived by providing better herd health, vaccination, and genetic selection. Future success come through farmer education, antimicrobial control and monitoring and global commitment, research, and cross-sector collaboration across the dairy value chain.

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Competing Interests

The authors should declare that there are no competing interests.

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