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Review Paper

Review of Promising Means to Decrease Antimicrobial Residues in Milk

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ABSTRACT

Milk is one among the major foods consumed worldwide. Beyond its nutritional value, it is economically important since there are more than 6 billion consumers for milk and milk products. The quality of milk determined by its components, microbial quality and now drug residues too. Antimicrobials used in animals as therapeutic, prophylactic and sometimes used as feed additives which causes drug residue in milk. If antibiotics are present beyond their maximum residue level (MRL), there will be health damage to the consumer ranging from allergic reactions to bacterial resistance. Some of the studies reported that the excessive use of antibiotics in animals to the appearance of super bacteria in humans. Above 60,000 tons of antibiotics are being used in livestock worldwide which is double the amount compared to human. Many times antibiotics are released unaltered, with full potential of antimicrobial activity. Methodologies or protocols to mitigate the antimicrobial residue in milk are detailed in this review. Since this antimicrobial residue (AMR) problem is worldwide threat, awareness among the milk producers and consumers must be created. Judicious usage methods of antimicrobials are detailed which must be entertained by the veterinarians. The modern tools with high sensitivity for quick and accurate detection of AMR at field level viz., CG-MS (gas chromatography coupled to mass spectroscopy), HPLC (High performance Liquid Chromatography), FT-NIR (Fourier Transform Near Infrared Spectroscopy) were discussed with their usage and drawbacks. Existing ethnoveterinary medicines to replace the antibiotics in the treatment of ailing dairy animals are reviewed and suggested the adoption practices for documented and established herbal practices. Alternating the antimicrobials novel bio-controlling agents like bacteriophages against antimicrobial resistant Staphylococcus may be intervened and various bio-controlling agents are discussed. To reduce the antibiotic usage, proper bio security measures are to be followed for avoiding the infections. Various biosecurity measures of dairy farming are given in this review, to be implemented by small, medium and large scale farmers. It is suggested that one of the major ways and means to reduce the AMR in milk is not procuring the milk with the contaminant of AMR. Milk of the animals which are in the treatment or withholding period of the antibiotic after treatment should not be included in the pooled milk. Policy makers should take the strong decision for procurement of milk to reduce the AMR in milk.

HIGHLIGHTS

- Antibiotic usage in animals is unavoidable which causes drug residues in milk and other livestock products.
- Excessive usage of antibiotics leads to appearance of superbacteria in humans.
- Antibiotic residues in milk is reduced by adopting good manufacturing practices in animal husbandry and management.
- Ethno - veterinary herbal treatment is promising way that alternating the use of antibiotics in treatment of ailing animals.
- Bio - controlling agents are best alternative to the antibiotics in treatment of mastitis.

Keywords: Antimicrobial residue, CG-MS, FT-NIR, milk, biosecurity, bio-controlling agent

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In veterinary practice, antibiotic usage is unavoidable, which is a driving force for antimicrobial residue in milk and other livestock products. High usage of drugs, incorrect dosages and treatment duration are the factors leading the complication of clinical condition. Some of the antibiotics are included in feeds as growth promoter and prophylactic treatment for prevailing condition.

According to UK prediction report, O'Neill, 2016, Drug-resistant infections will leads to death of extra 10 million people per year worldwide by 2050. The total cost of antimicrobial resistance on world Gross Domestic Product (GDP) will be $ 100 trillion (Thomas *et al.* 2015).

Antibiotic residues may inhibit the growth of cultures using in fermented milk products like curd, cheese and yoghurt. It is also causes the antibiotic resistance which is a major threat worldwide. Some of the recent studies reported that antibiotic residues like tetracycline are reabsorbed and retained in the body for a long time after administration, resulting in photosensitivity reaction with developed pigmentation of the nails. Similarly tetracycline-contaminated milk consumption may lead to the developments of permanent discoloration of the teeth in children (Navratilova *et al.* 2009) and azithromycin affected the human immune system and cardiovascular system (Lucas *et al.* 2010).

Under the "One health" concept, all relevant factors related to the issue such as animal, environment and human are required to be sort out. According to WHO guidelines, awareness and understanding about the antibiotic residue are must and to be created among the producers and public. There is a poor awareness about the withdrawal period of antibiotic residues and its stability in milk among the milk producers.

Veterinary drugs like penicillins, quinolones, macrolides, tetracyclines, sulfonamides, and trimethoprim are commonly used for symptoms diarrohea, mastitis, respiratory problems etc., Table 1 represents the maximum residue limit of various antibiotics in milk.

Antibiotic detection techniques

Now a days there is a increasing trend of consumer awareness that creates routine testing of milk for its quality and safety among farmers and industry. Since antimicrobial residue is the major health concern issue, there is a continuous research on qualitative milk screening tests and various chromatography-based techniques.

Table 1: Common drugs used in veterinary and their MRL in milk

Family of antibiotics	Antibiotics	MRL in milk (µg/L)
Penicillins	Amoxicillin	4
	Penicillin G	4
	Ampicillin	4
	Cloxacillin	30
	Dicloxacillin	30
	Nafcillin	30
	Oxacillin	30
Quinolones	Enrofloxacin	100
	Marbofloxacin	75
	Flumequine	50
Macrolides	Erythromycin	40
	Spiramycin	200
	Tilmicosin	50
	Tylosin	50
	Neospiramycin	200
Tetracyclines	Chlortetracycline	100
	Oxytetracycline+ 4-epi-oxytetracycline	100
	Tetracycline+ 4-epi-Tetracycline	100
Sulfonamides	Sulfadiazine	100
	Sulfapyridine	100
	Sulfamethoxypyridazine	100
	Sulfadoxine	100
	Sulfadimethoxine	100
	Sulfadimidine	100
	Sulfamonomethoxine	100
	Sulfamoxole	100
	Sulfaquinoxaline	100
	Sulfachloropyridazine	100
	Sulfathiazole	100
	Sulfamerazine	100
	Sulfamethoxazole	100
	Sulfacetamide	100
	Sulfisoxazole	100
	Trimethoprim	50
	Sulfaguanidine	100
	Sulfamethizole	100

Source: *Layada et al. 2016.*

Conventionally, qualitative antibiotic residue is detected in milk samples after proper incubation at particular temperature for a given period of time. If the test is negative, there will be bacterial growth and detected visually by opacity of the medium or by the color change of the pH indicator. This method is advantageous due to its low cost, easy to perform, and broad selection toward different antibiotics. But these tests may take time and lead to false negative or positive results.

Now, there are various techniques available to detect antibiotic residue in milk like immunoassay which provide results within 30 minutes. Chromatography is the technique widely used for quantitative detection of AMR but it needs expensive equipment and skilled manpower.

Kumar *et al.* (2021) using Dipicolinic acid test (DPA test) and strip test which was developed and patented by National Dairy Research Insitute, Karnal. This strip test is one step easy detection kit, involving the mechanism of color change of paper strip due to the germination of spores in milk. This is useful in detection of antibiotic residue in milk at farm itself. Luiz *et al.* (2018) detected the enrofloxacin, terramycin, penicillin, and ceftiofur hydrochloride in milk through Fourier transform near-infrared absorption spectroscopy (FT-NIR) jointly with Principal Component Analysis software even below the MRL limit. This method is rapid and high efficacy in analysis of antibiotic residues in milk.

Moharana *et al.* (2015) detected enrofloxacin residues using reverse phase HPLC with orthophosphoric acid, acetonitrile and methanol as mobile phase with a detection limit of 100 µg/L. Layada *et al.* (2016) compared the conventional Delvo P test and LC MS method and reported that more than 65% of non confirmed samples detected with antibiotic residues with more than prescribed limit by LC MS method. It is reported that, oxytetracycline had an average positive presence of 20 d in the evaluation of AMR in milk with quick detection kits and it confirms that the drug is not suitable for lactating cows.

Residues of penicillin G and kanamycin in milk were detected by newly developed competitive magnetic immunodetection (cMID) with an accuracy of 91-112%. Direct analysis of milk can be performed without further preparation steps of milk to detect pencillin in milk @4 $ng \cdot mL^{-1}$ by addition of antibody and for kanamycin detection, milk should be diluted threefold. Pietschmann *et al.* (2020).

Antibiotic residues reports in India

Moudgil *et al.* (2019) reported that out of 163 samples revealed in Punjab, 16 samples showed positive for antibiotic residues such as enrofloxacin (4.8%), oxytetracycline (3.0%), penicillin G (3.0%) and sulphamethoxazole (0.6%) residues. Oxytetracycline residues detected in 73% of individual animal milk samples collected from Hyderabad, India (Sudershan and Bhat (1995).

Kumar *et al.* (2021) found 40 samples out of 491 samples were positive for antibiotic residues in milk such as betalactam, tetracycline, sulphonamides, macrolides and quinolone. They found these residues in large and medium scale dairy farms as well as in small scale farmers in Assam and Hariyana state of India.

Kalla *et al.* (2015) reported in coastal areas of Andhra Pradesh that 18% of 300 raw milk samples showed antibiotic residues viz., penicillin, tetracycline and oxytetracycline. Moharana *et al.* (2015) reported 16.8% of samples positive for enrofloxacin residues in Tamil Nadu.

Kumaraswamy *et al.* (2018) screened raw milk samples and found betalactam, enrofloxacin and tetracycline residues. Betalactam and tetracycline residues were found in raw milk samples of Thrissur, reported by Lejaniya *et al.* (2017).

Kurjogi *et al.* (2019) identified azithromycin and tetracycline residues from two raw milk samples out of 11 samples at Karnataka state, India.

Nair *et al.* (2022) tested the 35 market milk samples (branded) and 33 samples reported positive for antibiotic residues such as betalactam, sulphonamide, gentamycin and tetracycline.

Rationale for antibiotic residues in milk

India ranked first in worldwide milk production and small scale dairy producers are playing major role in producing. Around 70 million households are depending dairy for their livelihood. They have only poor infrastructure, lack of awareness, irrational antibiotic usage, lack of knowledge about dosage, duration and withdrawal time for milking after treatment with antibiotics.

Kumar *et al.* (2021) studied the probable reasons for antibiotic residues combined with farmers, farm and milk samples. Farmer's knowledge, aptitude and practice over the antibiotics was examined in two states of North East India and on site farm examination and screening of milk samples for the presence of antibiotic residues. They reported that only 2% of the farmers knew the term "withdrawal period" and around 40 % of farmers only know the term antibiotic.

Limited detection facilities and poor monitoring system for antibiotic residues in milk at farm level is the primary reason. Further, there is a lack of awareness among the farmers about the effect of antibiotic residues in health as well as industry concern.

Antibiotics are to be used judiciously and the manufacturer's instructions to be followed. Disease condition of an animal alters the pharmacokinetics of the antibiotic drug. If an animal is sick, drug will be deposited in to the affected tissue for a long period of time, which increases the residues problem. It is the veterinarians responsibility to administer/prescribe the

Mitigating Steps for occurrence of antibiotic residues in milk

Government steps

Government of India is taking continuous efforts in regulating the use of antibiotics in animals such as National Policy for Containment of Antimicrobial Resistance, 2011, new category of schedule H drugs in the Drugs and Cosmetics Rules, 2013, Food Safety and Standards (Contaminants, Toxins and Residues) Amendment Regulations, 2017 and new tolerance of 103 antibiotics in meat, poultry, milk and fish (FSSAI, 2018).

Managemental practices

By adopting good manufacturing practices in animal husbandry and management, occurrence of antibiotic residues may be reduced in milk. Biosecurity measures at dairy farm and good managemental protocols help in prevention of disease. Isolation of new/sick animals for surveillance and treatment will help the spreading of disease in to herd. Sick animal should be milked after milking the healthy animals and it should not be mixed with pooled milk. Record maintenance about treatment, vaccination and deworming should be kept in regular manner so that withdrawal time may be followed in antibiotic treated animals, to prevent the antibiotic residues in milk. Monitoring of antibiotic residues in milk using rapid screening tests at the farm level should be done periodically.

Ethnoveterinary practices

In India, there are scientific reports evidenced that ethno-veterinary practices were successful in treating the ailing animals (Nair and Unnikrishnan, 2010; Nair 2019; Nair *et al.* 2017a, b, 2021; Nair and Punniamurthy 2002; Rana *et al.* 2021; Ngwisha *et al.* 2021; Dutta *et al.* 2020, 2022a; Shrestha *et al.* 2014).

Ethno-veterinary herbal treatment is promising way that alternating the antibiotics even in severe clinical conditions such as diarrhea, mastitis, foot and mouth disease, indigestion and bloat etc. Ethnoveterinary practices are being considered as best in efficacy as well as in cost wise.

Nair *et al.* (2022) revealed in his study that, 123 farmers out of 140 farmers who trained and practiced ethnoveterinary practices for their dairy animals had no antibiotic residues in their milk. The remaining 27 farmers were practicing ethnoveterinary combined with antibiotic treatment had antibiotic residues in their milk. This study involved various clinical conditions such as mastitis, udder pox, FMD, diarrohea, repeat breeding, bloat, indigestion and maggot wounds. This study strongly recommended that EVP had significant effect in antibiotic residues in milk.

While these studies suggest that some ethnoveterinary treatments may be effective in treating dairy animal health problems, further research is needed to determine their safety and efficacy to make them widely available knowledge. If this become wide popular and easy adoption, surely there will be half the way achieved in reduction of AMR in milk.

National Dairy Development Board, India launched Mastitis Control Popularisation Programme (MCPP) through ethnoveterinary treatment and currently engaged in 8 stated. Now this MCPP is doing beyond the mastitis, 29 other diseases treated with ethnoveterinary medicines.

Latest alternatives to antibiotics in treating mastitis

Bioresonance therapy

Bioresonance therapy can be used to treat mastitis. It is a non invasive complementary therapeutic practice which works on the basis of biological effects of electromagnetic fields. Generally, the inflamed cells emit altered electromagnetic waves owing to DNA damage. Measuring these waves can be used to diagnose the disease and at the same time, modifying these waves back to their original frequency may heal the condition. In human medicine, it is used for treating the patients with rheumatoid arthritis. Lyubimov *et al.* (2020) reported this bioresonance therapy in treating bovine mastitis. The resonant frequencies are though to inhibit the growth of mastitits pathogens.

Photodynamic Therapy

Photodynamic Therapy (PDT) is an effective method in treating mastitis caused by Gram-positive, Gram negative, yeast and fungal bacteria. The mechanism of PDT is the activation of a non-toxic photosensitizer that, when exposed to light at a suitable wavelength and energy density, generates reactive oxygen species such as free radicals and singlet oxygen, which, when in contact with microorganisms, can modify bacterial DNA and cell membrane, resulting in microbial death.

Moreira *et al.* (2018) used photodynamic therapy for treating subclinical mastitis with application of 1.0 mL of 2.5% toluidine blue photosensitizer followed by LED irradiation at λ = 635 nm. They observed the significant reduction of the bacterial load of *Streptococcus dysgalactiae* and coagulase-negative Staphylococcus in photodynamic treated cows.

Biocontrolling Agents

Researchers isolated and characterized a bacteriophage (L1) with activity against *Staphylococcus aureus*, a common mastitis-causing bacteria. In a field trial, the affected cows were treated with L1 and observed a significant reduction in *S. aureus* counts in the milk samples of the treated cows compared to the control group (Wang *et al.* 2019). Oliveira and Sillankorva, (2018) evaluated the efficacy of a group of bacteriophages against several mastitis-causing bacteria including *Staphylococcus aureus, Streptococcus uberis,* and *Escherichia coli*. Further they identified that the treatment was well-tolerated by the animals. Further, some of the recent studies demonstrated that bacteriophages are best alternative to the antiobiotics in treatment of mastitis (Zou *et al.* 2022; Hidalgo-Cantabrana, 2021; Sisto *et al.* 2021).

Most of the studies on phage-based mastitis treatment have been conducted in vitro or in small-scale field trials. While these studies have shown some efficacy in reducing bacterial counts in milk samples and improving cow health, larger clinical trials are needed to determine the safety and effectiveness of these treatments in clinical settings.

It's also important to note that regulatory approval for phage-based treatments can be challenging due to the complexity of phage biology, variability between phage strains, and concerns over safety and efficacy. Then this type of phage treatment may be the promising way of reducing the antimicrobial residues in milk

CONCLUSION

The problem of AMR in milk is a worldwide threat to the consumers as well as to the industry. Reduction of AMR is in the hand of farmers in terms of production of good quality milk; veterinarians should follow the principles of antibiotic usage, withdrawal time etc., policy makers to adopt alternate strategies to treat the ailment of animals and milk procurement policy; subject specialized extension worker in creating awareness among the farmers/consumers about the significance of AMR in milk

REFERENCES

Chambers HF. Goodman and Gilman's the pharmacological basis of therapeutics. 11th ed, New York: McGraw-Hill Medical Publishing Division:

Cristina M., Melo R.T., Mendonça E.P., Coelho L.R. and Rossi D.A. 2010. Use of ceftiofur in dairy cows and their excretion in milk. *PUBVET.,* **4**: 931–937.

Hidalgo-Cantabrana, C., Goh, Y.J., Barrangou, R. and Klaenhammer, T.R. 2021. *Streptococcus thermophilus* strains as a natural biocontrol against mastitis pathogens. *J. Appl. Microb.,* **131**(5): 2525-2537.

Kumar, N., Sharma, G., Leahy, E., Shome, B.R., Bandyopadhyay, S., Deka, R.P., Shome, R., Dey, T.K.

and Lindahl, J.F. 2021. Understanding Antibiotic Usage on Small-Scale Dairy Farms in the Indian States of Assam and Haryana Using a Mixed-Methods Approach—Outcomes and Challenges. *Antibiotics*, **10**: 1124.

Kurjogi, M., Issa Mohammad, Y.H., Alghamdi, S., Abdelrahman, M., Satapute, P. and Jogaiah, S. 2019. Detection and determination of stability of the antibiotic residues in cow's milk. *PLoS ONE*, **14**(10): e0223475.

Layada, S., Benouareth, D.E., Coucke, W. *et al.* 2016. Assessment of antibiotic residues in commercial and farm milk collected in the region of Guelma (Algeria). *Food Contamination*, **3**(19).

Lubimov, Victor & Romanov, Dmitry & Tsoi, Yuri & Ziganshin, Bulat & Sitdikov, Farit. 2020. Results of application of frequency resonance therapy for treatment of cow mastitis. *BIO Web of Conferences*, **17**. 00254. 10.1051/bioconf/20201700254.

Lucas, M.F., Errecalde, J.O. and Mestorino, N. 2010. Pharmacokinetics of azithromycin in lactating dairy cows with subclinical mastitis caused by *Staphylococcus aureus*. *J. Vet. and Pharmacology Therapeutics*, **33**(2): 132–40.

Luiz, L.D.C., Bell, M.J.V., Rocha, R.A.D., Leal, N.L. and Anjos, V.D.C.D. 2018. Detection of veterinary antimicrobial residues in milk through near-infrared absorption spectroscopy. *Journal of Spectroscopy*.

Moharana, B., Venkatesh, P.K., Preetha, S.P. and Selvasubramanian, S. 2015. Quantification of enrofloxacin residues in milk sample using RPHPLC. *World J. Pharm. Pharm. Sci.*, **4**: 1443–1450.

Moudgil, P., Bedi, J.S., Aulakh, R.S. and Gill, J.P.S. 2019. Antibiotic residues and mycotoxins in raw milk in Punjab (India): a rising concern for food safety. *J. Food Sci. Technol.*, **56**(11): 5146-5151.

Navratilova, P., Borkovkova, I., Drackova, M., Janstova, B. and Vorlova, L. 2009. Occurrence of tetracycline, chlortetracyclineand oxytetracycline residues in raw cow's milk. *Czech J. Food Sci.*, **27**(5): 379–385.

O'Neill, J. 2016. Review of antimicrobial resistance. Tackling drug-resistant infections globally: Final report and recommendations 19th May 2016, Government of the United Kingdom.

Oliveira, M. and Sillankorva, S. 2018. Bacteriophages as an alternative to antibiotic treatment for the prevention of bovine mastitis. *J. Appl. Microb.*, **125**(6): 1542-1551.

Pietschmann, J., Dittmann, D., Spiegel, H., Krause, H.J. and Schröper, F. 2020. A Novel Method for Antibiotic Detection in Milk Based on Competitive Magnetic Immunodetection. *Foods*, **9**(12): 1773.

Shrestha, S., Kaushik, P. and Dhakal, S. 2014. Ethnomedicinal knowledge, diversity and equity: A case study from Nepal Himalaya. *J. Ethnopharmacology*, **157**: 224-230.

Sisto, A., Martini, M. and Caprioli, A. 2021. A novel approach to the control of bovine mastitis: use of lytic bacteriophages against coagulase-negative staphylococci. *Frontiers in Vet. Sci.*, **8**: 678829.

Thomas, P., Van Boeckel, Charles Brower, Marius Gilbert, Bryyan, T., Grenfell Simon, A. *et al*. 2015. Global trends in antimicrobial use in food animals. *PNAS.*, **112**(18): 5649-5654.

Wang, J., Hu, B., Xu, M., Yan, X., Zhang, X., Zhang, H., Hao, Y. and Wang, R. 2019. Characterization and field application of a novel lytic bacteriophage, L1, against *Streptococcus agalactiae* causing bovine mastitis. *J. Dairy Sci.*, **102**(7): 6342-6351.

Zou, J., Meng, Y., Wang, J., Wang, Y., Zhang, R., Zhu, Y. and Cui, Z. 2022. Bacteriophage therapy against *Escherichia coli*-induced bovine mastitis: efficacy evaluation *in vitro* and *in vivo*. *J. Dairy Sci.*, **105**(1): 444-452.