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Review Article

PREVENTION STRATEGY FOR ANTIMICROBIAL RESISTANCE DEVELOPMENT IN BACTERIA THROUGH MILK AND CONSERVATION OF ANTIBIOTIC SENSITIVITY

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Residue of some antibacterial drugs persist at a lower concentration for prolonged time in milk of cows, buffaloes and goats after treatment of bacterial infections by antibacterial drugs following repeated or single dosing. Repeated dosing of antimicrobial agent is required to maintain therapeutic concentration for desirable period to cure mastitis and other bacterial infections. However, prolonged persistence of the antibiotic residue selectively preserves the resistant microorganism which triggers antimicrobial resistance development. Our recommended herbal preparations should be applied in milk producing animals towards production of safe milk as it is consumed by a large population and suckling animals. These herbal preparations have the potential to conserve antibiotic sensitivity which would keep the antibacterial drugs effective towards successful therapy of different bacterial infections in animals and human beings.

Key words: Antibiotic residue, Antimicrobial resistance, Dairy animals, Herbal drugs, Persistence

Residue of some antibacterial drugs persist at a lower concentration for prolonged time in milk of cows, buffaloes and goats after treatment of bacterial infections by antibacterial drugs following repeated or even after single dosing (Ismail, 2005; Stolker *et al.*, 2008) which increases the probability of antibiotic resistance development in bacteria. These resistant bacteria are being developed upon exposure to sub MIC (minimum inhibitory concentration) level of antibiotics may spread to different animals as well as humans resulting in public health hazards (Vasavada,1998; Wistrand-Yuen *et al.*, 2018). Sub-lethal levels of β -lactam antibiotics, aminoglycosides, and fluoroquinolones result in increased

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mutation frequency in *Pseudomonas aeruginosa* causing resistance development against these antibiotics (Nair *et al.*, 2013). Methicillin and vancomycin resistant *Staphylococcus aureus* (Normanno *et al.*, 2007; Sasidharan *et al.*, 2011) oxacillin and penicillin resistant *Listeria monocytogenes* (Harakeh *et al.*, 2009) and multi drug resistant *Listeria* species (Rahimi *et al.*, 2010) were detected in milk and milk products. Ampicillin, vancomycin, and ciprofloxacin resistant *Escherichia coli*, *Salmonella* spp. and *Klebsiella* spp. were also isolated from raw milk, pasteurized milk and yogurt samples (Marjan *et al.*, 2014). Administration of antibacterial drugs to dairy animals suffering from chronic mastitis may also lead to lower concentration of antibacterial drugs in milk due to fibrosis (Dash *et al.*, 2019). There are over 264 million dairy cow worldwide, producing nearly 600 million tonnes of milk every year. The average rate of milk yield is 2,200 litres per cow (FAO STAT, 2012). Around 12.5% of world's cattle population is present in India with a total cow population of 122.9 million and out of which 19.42 million is exotic/crossbred milch cattle (Livestock census, 2007). Occurrence of mastitis in dairy animals stands at first position as mastitis prevalence had been observed to be more than 90% in crossbred dairy cows (Sharma, 2003). Mastitis is the inflammation of the mammary gland which is manifested by physio-chemical and microbial alterations in the milk with pathological lesions in the udder. The primary reservoir of contagious pathogens is mammary gland whereas contaminated surroundings are one of the main sources of microorganisms causing mastitis (Hillerton and Berry, 2005). *Streptococcus agalactiae, Staphylococcus aureus* and *Mycoplasma* spp. are reported as dominant infecting microorganisms. Streptococci other than *Streptococcus agalactiae* such as *Streptococcus uberis* and enterococci are typical environmental pathogens causing mastitis (Kluytmans *et al.*, 1997). The most prevalent causative organism is *Staphylococcus aureus* that causes acute suppurative, gangrenous, or chronic mastitis. Mastitis generally appears in clinical and sub-clinical forms (Radostits *et al.*, 2000). Incidence of sub-clinical mastitis is 15-40 times higher than clinical mastitis. *Staphylococcus aureus* is also a source of food borne diseases (FBD) imposing estimated 241,000 illnesses per year in USA (Scallan *et al.*, 2011). FBD involves great economic burden due to lost productivity, health care and management in the United States (Scharff, 2012; Byrd-Bredbenner *et al.*, 2013). *S. aureus* produces a variety of toxins namely Staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) (Balaban and Rasooly, 2000; Argudín *et al.*, 2010). Pyrogenic toxins are responsible for immune suppression along with nonspecific T-cell proliferation (Le Loir *et al.*, 2003). *S. aureus* is also a common mastitis causing microorganism in goats (De Buyser *et al.*, 1987). SEC was the predominant toxin isolated from Staphylococcal mastitis milk samples from sheep, goats and cattle (Scherrer *et al.*, 2004). The sources of enterotoxin producing *S. aureus* were goat's milk, udder and teats (Valle *et al.*, 1990; Foschino *et al.*, 2002). Six SFD outbreaks occurred in France in 2009 by SEE from soft cheese prepared from unpasteurized milk (Ostyn *et al.*, 2010).

Public health hazards through milk

To address the global problem of mastitis and presence of milk antibiotic, some therapeutic strategies have been developed which can be applied in a rational manner in field condition and dairy farms. Antibiotic is commonly employed for treatment of mastitis and the effectiveness of antibacterial drugs can be maximized by maintaining minimum inhibitory concentration (MIC) for a desirable period (Sawant *et al*., 2005; Cagnardi *et al*., 2010). Antibacterial drugs are useful to treat the bacterial infection but they cannot protect the glands from tissue damage. Repeated administration of antimicrobial agent is also required to maintain MIC for an adequate period to cure mastitis which is not often cost effective. Besides, the animals sometime suffer from systemic infection due to the spread of the microorganism from the infected mammary gland. Intramammary administration is also adopted mastitis treatment but, intramammary administration of most of the antimicrobial agents was suspected to cause mammary gland tissue damage. The imbalance of innate antioxidant system and reactive oxygen species leads to oxidative stress making the mammary gland more prone to tissue damage that also results in marked reduction in milk production. Persistence of antibacterial drugs in milk enhanced probability of antimicrobial resistance development in bacteria. Milk is a common food item as it is a balanced diet (FAO Statistics Division, 2007). However, prolonged persistence of the antibiotics selectively preserves the resistant microorganism which triggers antimicrobial resistance development. On the other hand, milk is considered as nutrient rich media which acts as favourable environment for growth and multiplication of those resistant microorganisms. The incidence of mastitis was reported in women during lactation which may go up to 33%. Higher incidence of women mastitis had been reported from Australia and the major causative organism was *Staphylococcus aureus* (Fetherston, 1997). Selection of antimicrobial agent and maintenance of therapeutic concentration are the major challenges in antibiotic therapy of mastitis. New cephalosporins are preferred for mastitis therapy in dairy due to beta-lactamase resistance, spectrum of activity and lack of toxicity (Mattila-Sandholm *et al*., 1990). Parenteral administration of ceftriaxone and ceftizoxime has been a common practice for treatment of bacterial infections including mastitis. However, ceftizoxime was excreted for comparatively longer period through milk of cows, goats and buffaloes (Sar *et al*., 2006; 2008; 2010; 2011 and 2013). Mastitis is still remaining as a costly dreadful disease in livestock in the world due to frequent failure in prevention and treatment. The major reasons are unplanned therapy or lack of strategy for prevention, poor management, unhygienic environment and treatment without proper diagnosis. In many developing and underdeveloped countries, the infrastructures needed for diagnosis of different stages of mastitis are not available. Failure of proper diagnosis many times results in unsuccessful treatment. Even antibiotic sensitivity test is not done in most of the cases, sometimes due to lack of facilities and many times due to severity of the disease giving no scope to wait for antibiotic sensitivity test result. In that situation, veterinarians or medical doctors

have to administer main drug with supportive drugs empirically. As a result, it leads to aggravation of mastitis cases. In case of dairy animals, milk production is badly affected due to treatment failure. Health of young suckling animals and milk consumers are also compromised. On the other hand, improper and indiscriminate use of antimicrobial drugs associated with their harmful drug interactions result in adverse effects of drugs in mastitis affected animals as well as milk consumers. Another great concern of the present conventional therapy of mastitis is the antimicrobial resistance development in the microorganisms that can cause serious microbial infections in humans especially in children as they consume milk on a regular basis. Long term use of single or multiple antimicrobial (combined) drugs leads to antimicrobial drug residues in milk and dairy products and also causes selection pressure helping the microorganisms to resist these drugs. Sometimes, these resistant microorganisms spread through various routes and jump from one species of animal to another animal species. Animals and humans also suffer from infections of resistant microorganisms due to lack of antibiotic stewardship and antimicrobial drug policy particularly for veterinary drugs in almost all countries. All these problems associated with the present conventional mastitis therapy are imposing threats to public and animal health.

Approaches for conservation of antibiotic sensitivity

Therefore, new approaches are the needs of the time to address the burning global problem of antibiotic resistant microorganisms spreading through consumption of milk and dairy products. Some supportive herbal therapies developed by our team, can be used as adjunct to antibacterial drugs to protect the animal from oxidative stress, inflammation and related patho-physiological changes. It was found that *Oscimum sanctum* leaf juice may be useful as supportive therapy to intravenous ceftriaxone for treatment of caprine Staphylococcal chronic mastitis due to its potent bioavailability enhancing and antioxidant properties (Dash *et al.*, 2016). *Bauhinia variegata* L. bark powder following repeated oral dosing, once daily for 14 days, showed anti fibrotic, antibacterial, anti-inflammatory and antioxidant activities in chronic Stapylococcal mastitis which may be recommended as supportive therapy with antimicrobial drug (Dash *et al.*, 2014). Generally, broad spectrum antibacterial drugs are preferred over narrow spectrum drugs as mastitis is often caused by multiple numbers of organisms. Use of single antibiotic or combination following repeated dosing may result in undesired persistence of antibiotic residues in milk/mammary gland increasing chances of development of antimicrobial resistance and other pathological complexities. A broad-spectrum antibiotic i.e. ceftriaxone was reported to be effective in mastitis as a single dose therapy in cows and goats (Sar *et al.*, 2006; 2010). Another antibiotic having structural similarity with ceftriaxone i.e. ceftizoxime was also effective for buffalo mastitis (Kumar *et al.*, 2016). The herbal preparations like *Ocimum sanctum* (tulsi) herbal extract or *Bauhinia variegata* (shetkanchan) bark powder can be recommended as supportive therapy with

single dose of intravenous ceftriaxone/ ceftizoxime in mastitis in cows, buffaloes and goats. When cattle are treated with antibiotics, their milk retains the antibacterial drug residue for a significant time that may be a triggering factor antimicrobial resistance (AMR) development. It was reported that a commercial herbal drug (Fibrosin®) when given with antibiotic has the potential to prevent antimicrobial resistance in causative organisms (Sar *et al*., 2018). Mastitis, a prevalent disease in cows, goats and buffaloes, is not only a major concern for the milk production but also a public health menace. Ceftriaxone gets converted into an active metabolite called ceftizoxime in cows and goats, another third generation cephalosporin that excreted for longer time in their milk. Fibrosin® is recommended to increase the bioavailability of ceftizoxime (metabolite) in milk and also to eliminate it at a much faster rate compared to control goats that did not receive the commercial herbal drug (Sar *et al*., 2018). Therefore, our recommended herbal supportive therapies should be applied in milk producing animals towards production of safe milk as it is consumed by a large population in the world. These herbal preparations returned milk alkaline phosphatase and catalase activity in mastitic goats to normal level and increased lactoperoxidase (antimicrobial system in milk) activity significantly (Sar *et al*., 2012; Dash *et al*., 2014; 2016).

Conclusion

Therefore, the commercial herbal drug and other mentioned herbal preparations are recommended to decrease persistence of these cephalosporins like ceftriaxone and ceftizoxime following their parenteral administration. These herbal preparations can conserve antibiotic sensitivity which would keep the antibacterial drugs effective towards successful therapy of different bacterial infections as these drugs are used both in humans and animals.

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