**Future and role of bacteriocins as antimicrobials in food safety applications**

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***Background***: Overuse and misuse of conventional antimicrobials in animals, food and humans has led to new strains of microbes with increasing levels of antimicrobial resistance (AMR). According to the World Health Organisation (WHO), AMR is within the top 10 public health threats as well as food security. Thus, it is a priority across the globe. AMR infections cause 700000 deaths worldwide per annum, and this is predicted to rise to 50 million by 2050 [1]. However, predictive statistical models suggest that these values could be much higher. The additional cost to health systems is approximately US$14001 per patient as a result of prolonged hospital stays and the need for more expensive and intensive care. The estimated economic output loss as a result of AMR is 1% of gross domestic product per year, rising to between 5 - 7% for developing counties by 2050 [2]. The projected AMR cost is expected to rise from US$300 billion to over US$ 1 trillion by 2050. The criticality of AMR is such that it has been explicitly mentioned in Sustainable Development Goal (SDG) and impacts the achievements of several other SDGs [3]. Microbial infections are very common (there are more than 1 million cases per year) in India one of the major complications leading to antimicrobial resistance. Antimicrobial resistance transmission via food-borne pathogens is a major concern all over the world [4]. The government of India is very much aware of it and realizes that microbial infections are environmental as well as development issue. The Indian Public Health Authorities released their National Action Plan on Antimicrobial Resistance in 2018 at World Health Assembly in Geneva in May [5]. The National Action Plan on Antimicrobial Resistance covers a wide range of themes and factors that drive antimicrobial resistance and clearly outlines all the challenges that need to be tackled. Therefore, government is fully committed to manage this issue so that country is protected from its adverse effects and the growth path remains stable.

***The problem***: In modern farming practice (aquaculture, livestock and crop production), antimicrobials are widely administered for therapy as well as metaphylaxis, as such these are mass administered via feed or water. Up to 80% of total antibiotic consumption is administered to animals in some countries [6]. Antibiotics consumed by animals are not completely absorbed in the gut and excreted out with waste matter that is used to produce manure. This is used to fertilise soil resulting in transfer of antibiotic resistant genes (ARGs) to agricultural soil ecosystem and then onto plants [7]. A study by the European Centre for Disease Prevention and Control, the European Food Safety Authority and the European Medicines Agency in 2015 concluded that antimicrobial consumption results in bacterial resistance in both humans and animals. Fish, food animals and vegetables are reported to be large reservoirs of antibiotic-resistant bacteria (ARB) [8]. Furthermore, antimicrobials are also added to food as they play an important role in preventing food spoilage by suppressing the growth of food pathogens. Whilst these are added as preservatives, they contribute to the overexposure of antimicrobials and thus accelerate AMR spread within and along the food chain. ARB in food, in particular minimally processed food, may not effectively kill the bacteria and this results in food spoilage and/or cause food poisoning, which might not be treated by available antimicrobials as a consequence of AMR. The WHO estimates that 1 in 10 people fall ill from eating contaminated food with 420 000 global deaths per year [9, 10].

Antimicrobials are consumed or added as preservatives at various stages of the food chain. However, this results in widespread antimicrobial exposure, as it passes along the food chain through different ecosystems, and thus contributes to the spread of AMR. Resistance of significantly concerning foodborne bacteria to some antibiotics, such as *Campylobacter, L.* *monocytogenes and Salmonella* amongst others, has been reported [11, 12. It is well known that the accidental discovery of the antibiotic penicillin revolutionised modern day medicine. A large number of antibiotics and other antimicrobials have since been developed to manage or treat life-threatening bacterial, parasitic, fungal or viral infections. Over time, however, these pathogens develop resistance to antibiotics, rendering the antimicrobials ineffective. Primarily AMR has been overcome by the discovery of new drugs. There has been a concerted effort in the continual discovery of new antimicrobials, however since 1987 no new classes of antibiotics have been discovered and the quantity of antibacterial agents approved in recent times and those under clinical investigation are not sufficient to challenge the rise and spread of AMR [13]. In particular there are insufficient drugs to target the WHO’s priority Gram-negative pathogens, ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.*) [14]. In reference to food spoilage specifically, aside from AMR, antimicrobials that are otherwise apparently effective have shown to be much less effective against *Listeria innocua* in one study, requiring doses over 100 times greater, in a complex food matrix [15].

***The need*:** There is, therefore, an imminent necessity to prolong the lifespan of current antimicrobials and control the exposure of animals, crop, food and humans to antimicrobials in order to remain ahead of the AMR curve. In doing so, this reduces the urgency for the discovery, development and manufacture of new drugs. One way of reducing exposure is through efficient targeted delivery, aka smart delivery, of antimicrobials to minimally processed food.

***State of the art:*** AMR is a complex problem and as such requires a multi-sectoral approach. In 2017 the European Commission adopted their AMR action plan with a One Health Action Plan to address AMR in humans, animals and the environment [16]. The WHO, Food and Agriculture (FAO) and Animal Health (OIE) agencies have collaborated over several years on reducing antimicrobial administration in livestock and have drawn research and policy options to decrease antibiotic use in the food chain [17]. Strategies are based on slowing down and where possible reversing AMR through smarter use of antimicrobials and employing robust surveillance systems to monitor AMR and research and development activity. However, this is reliant on local resource capability.

***Which antimicrobials are currently deemed effective?*** With the low rate of new antibiotic discovery, the WHO has placed an emphasis on novel therapeutics. From a basic scientific strategy perspective, a potential new class of natural antibiotics are antimicrobial peptides (AMPs), in particular bacteriocins (nisin, pediocin, lacticin). AMPs are produced in nature by ribosomal translation of mRNA in all species. Notably, bacteria also synthesise AMPs non-ribosomally to inhibit or kill other microorganisms. Nisin has been used as bio-preservatives in various foods, they are active against a range of food pathogenic and spoilage bacteria due to their active range of pH values and resistance to high temperatures. Nisinis classified as a class-I-a bacteriocin, also called lantibiotic and is the most commercially important and characterised bacteriocin [18]. Pediocin is a class II-a bacteriocin that is produced by *Pediococcus spp*. Pediocin has been shown to be more effective than nisin against some food-borne pathogens such as *L. monocytogenes*, *S. aureus, Pseudomonas* and *E. coli* [19].

Lacticin is a two-component lantibiotic isolated from an Irish kefir grain used for making buttermilk. Lacticin exhibits antimicrobial activity against a wide range of food-borne pathogens and food spoilage bacteria in addition to other lactic acid bacteria (LAB) [20].

***What are the concerns?*** For humans and animals, antimicrobial drugs are traditionally delivered by oral or parenteral routes as either tablets, capsules or intravenously. Topical antimicrobial formulations (creams, ointments or gels) may also be used for skin infections. In order to treat minimally processed food, the food is either dipped, sprayed or coated with the antimicrobial prior to packaging. The main reason for the different routes and systems is the need to maximise the bioavailability of a drug as this has a significant influence over its efficiency. Depending on the type of drug, bioavailability is adversely influenced by a number of factors such as low solubility, low permeability or enzymatic degradation for example. However, limitations, such as the uncontrolled interactions of bacteriocins with food components, proteolytic degradation and electrostatic repulsion, challenge their use as food bio-preservatives resulting in high antimicrobial concentrations to be administered. Similarly, bacteriophages have a low tolerance to differences in environmental conditions such as physico-chemical properties and temperature as well as method of application. The need to maximise bioavailablity and ensure adequate eradication of microorganism, results in antimicrobial “over dosing” which therefore contributes to excess antimicrobial exposure for the various ecosystems.

***Have the concerns been addressed effectively?*** Formulation technologies, such as various nanoparticles and carbohydrate-based structures, have been explored to protect antimicrobials from degradation or aggregation, whilst enhancing solubility, permeability and specificity. In doing so, this would increase the antimicrobial efficacy and maximise bioavailability without over dosing. The potential of inorganic nanoparticles of metals, such as gold, silver, zinc and copper, to prevent AMR has also been reported [21]. However, nanoparticles from metals and inorganic sources have limitations such as low clearance rates, minimal scope for surface modification and toxicity effects in humans as well as the environment. Nano-carriers based on synthetic polymers, such as polylactic acid and polylactic-co-glycolic acid also exhibited limitations, such as aggregation of the encapsulated biomolecules.Liposome carriers were shown to have poor drug encapsulation efficiency, low stability and high rates of release.Similarly, whist there has been progress with bio-based carriers there are still limitations [22]. For bacteriocin encapsulation, the production of yeast derived microparticles requires organic solvents or energy intensive approaches. The encapsulation efficiency of nanoliposomes was low and these formed an unstable encapsulation network. Nanomicelles proved to be unstable in a complex biological matrix and the fabrication of nanostructured lipid carriers requires costly pure oils to produce blends [23]. Nanotechnology may overcome this problem by [encapsulation](https://www.sciencedirect.com/topics/chemical-engineering/encapsulation) of antimicrobials into carbohydrate coated plant based protein nanoparticles, enhancing their efficacy and stability with specific targeting due to presence of polysaccharides onto the surface of proteins to combat antimicrobial resistance in food-borne pathogens. The use of [polysaccharides](https://www.sciencedirect.com/topics/chemical-engineering/polysaccharide) in protein nanoparticles can be an alternative for the controlled release of antimicrobials. Protein nanocomposites amalgamate the advantages of nano-sized structures with favorable characteristics of biomolecules. Proteins are biocompatible, biodegradable and abundant in nature. The amphiphilic nature and surface-active properties confer proteins their functional attributes, making them of great importance for the food industry as they can be applied into different products as emulsifiers, foaming or gelling agents [24]. Based on lower costs, health and religious, moral or environmental concerns related to the consumption of animal-based ingredients, Consumer trends towards plant-based foods and protein ingredients. Therefore, nanoformulated bacteriocin encapsulated plant-based protein-polysaccharide conjugates could be a biodegradable and eco-friendly solution to tackle this challenge.

However, during extraction, processing or storage, proteins can undergo partial or complete denaturation that can eventually affect their intended purpose and functionality. The different characteristics of proteins, such as their functional, surface and rheological properties, will directly impact on the sensory characteristics of food products, such as texture, aroma, flavor and appearance. Nano-conjugation of proteins will improve the functional properties compared to those of the native protein. The solubility and other functional properties of plant proteins have been subsequently improved through the conjugation with carbohydrates. Protein-carbohydrate conjugates can increase the thermal stability, and improved solubility, foaming, gelling and emulsifying properties, relative to the protein alone. These improvements will broaden the use of proteins to products that require severe processing conditions (broader pH range, higher temperatures) and to the development of tailored protein- carbohydrate conjugates for specific uses. The impact of conjugation on the nutritional quality of the proteins, as well as other uses in the food and pharmaceutical sectors, such as antimicrobial, antioxidant and nano-encapsulation of compounds for delivery. A very few reports on [encapsulation](https://www.sciencedirect.com/topics/chemical-engineering/encapsulation) of antimicrobials into carbohydrate coated protein nanoparticles, for improved capabilities in the food and nutrition field for their application in delivery systems, packaging, food safety and security has been reported [25].

Unlike free bacteriocins, nano-conjugated bacteriocins showed better stability and a wider range of antimicrobial action. Ultimately, nanotechnological approaches provide an attractive option for antimicrobial peptides to be developed on an industrial scale. Conjugation with protein–polysaccharide nanocomposite will enhance the antimicrobial activity of bacteriocins and, will also protect them from proteolytic degradation and extend shelf life of food product.

***Futuristic scope***: A set back is that the interaction mechanisms occurring in bacteriocin nanoconjugates responsible for strain inhibition is not fully understood as yet. Research in this field is in early stage and it is anticipated that progress can be made in the near future. To realize the true potential of bacteriocin- nanoconjugates for their application in food preservation, there is a need for understanding the interaction mechanisms between different bacteriocins and nanoconjugates and further, bacteriocin capsulated nanoconjugate interaction with pathogen cell wall. We are pretty hopeful that scientific efforts in future will surely help to develop safe and effective bacteriocin nanoconjugate approach for food bio-preservation and food safety application which will in turn reduce the antimicrobial resistance problem which has to be solved as soon as possible to save mankind.

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