Meat safety and quality: a biological approach

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Summary Safe, high-quality meat that is minimally processed, containing few added chemicals, is what is desired by the modern consumer. Biological approaches to meat safety appear more natural and, thus, are more readily acceptable. In this review, we examine biological approaches for meat safety and quality in the preharvest animal as well as during slaughter and in the post-harvest processing of the meat product. Biological components, including probiotics (Direct-Fed Microbials), vaccines, bacteriocins and lytic bacteriophage, are important components of a comprehensive approach to meat safety and quality.

Keywords Bacteriocins/colicins/microcins, bacteriophages, competitive exclusion, hurdle technology, preharvest and post-harvest meat safety and quality, probiotics/prebiotics/synbiotics/direct-fed microbials, protective cultures.

Introduction

The safety and quality of the food supply is of primary concern worldwide. The Centers for Disease Control and Prevention (CDC, USA) estimates that each year one in six Americans (about 48 million people) become ill, 128 000 are hospitalised, and 3000 die of food-borne diseases (Centers for Disease Control & Prevention, 2018). In 2017, the European Union (EU) member states collectively reported 5079 food-borne and waterborne outbreaks (43 400 cases). Six hundred and forty-two of the outbreaks were considered ‘strong-evidence’ food-borne outbreaks, with 60% of those (~385) attributed to food of animal origin, meat and meat products being the food most frequently involved (European Food Safety Authority & European Centre for Disease Prevention & Control (EFSA-ECDC), 2018). Despite multiple innovations in food biotechnology, modern methodologies, and the implementation of the Hazard Analysis Critical Control Points management system (HACCP) (U.S. Food & Drug Administration, 2018a; U.S. Food & Drug Administration, 2018b) food-borne outbreaks still happen. For this reason, researchers are constantly searching for solutions to prevent food contamination by food-borne pathogens. All stages of meat production, from farm to fork, are being investigated to find potential solutions to this worldwide problem. This would include steps taken on the farm where the animal is raised, during transport of animal, at the stockyard and packing plant, for carcass handling, further processing, distribution, at the food service and retail level, and preparation by the consumer. This review is concerned with the steps in this process where biological approaches could be most likely to yield enhanced results.

As consumers become more health conscious, they seek meat products that are minimally processed. Often consumers associate healthfulness of a food with it being ‘natural’ (Chambers et al., 2019). They consider safety a given and want their food to retain its familiar properties but reject excess chemical additives and antibiotics. For example, nitrites used in cured meats react with amines under high heat to form nitrosamines, which have been linked to cancer. However, nitrites are important since they inhibit Clostridium botulinum and contribute to the cured meat flavour and the pink colour of cured meat. In addition, nitrites play necessary roles in human physiology and are found in significant quantities in select vegetables (celery, spinach, broccoli, lettuce, etc.) (Sindelar & Milkowski, 2012; Iammarino et al., 2014). Methods have been developed using gas chromatography to quantify nitrates and nitrites (Luckovitch & Pagliano, 2020) and nitrosamines (Iammarino et al., 2020) in meat samples to assist in tracking the levels of these potential hazards, keeping them at a safer level for the consumer. Indeed, Iammarino et al. found levels of nitrosamine in meat even before it had been cooked.

Another concern is the use of subtherapeutic antibiotic regimens, partly responsible for emergence of antibiotic-resistant microorganisms. Many consumers

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are also wary of irradiated foods because of their lack of familiarity with the name and process, a problem that accurate consumer education may alleviate (Feng et al., 2019). Alternative safety measures need to be taken that are not rejected by the health-conscious consumer to provide the safe, high-quality meat product that is expected. Biological approaches have the advantage of being marketable as ‘natural’ and are more readily acceptable to the consumer to provide the safety they expect. Approaches we examine over several stops in the ‘food to fork’ process include probiotics/prebiotics/synbiotics, competitive exclusion, bacteriocins/colicins, bacteriophages, immunisation, carcass washing, fermentation, protective cultures and any combination of the above, a method known as ‘hurdle technology’.

The most critical element in keeping meat safe is sanitation at every step. This all starts on the farm. The reduction of pathogens on the farm is vital in decreasing the probability of pathogen presence in the animal which also reduces environmental pollution. This, in turn, reduces water and produce contamination as well as contamination of the meat itself (Sofos, 2008). In a perfect world, animal facilities (pastures, holding facilities, transport trailers, stockyards, etc.) would be kept pathogen free. Animals exhibiting any health issues would be held separately and treated appropriately. Animals would be tested regularly, and any found shedding pathogens would be given appropriate care. All feed materials and water would be pathogen free. But this is not a perfect world. Pathogens enter the herd through the air, the soil, contaminated feed and water, or even through wild animals including rodents and birds sharing their pasture. Animals become stressed, making them more susceptible to infection. Some become asymptomatic carriers, possibly even super shedders, who contaminate the holding areas and all other animals with which they share the space. Often livestock carry known human pathogens, nonpathogenic to the livestock, with the potential of these pathogens entering the food stream and infecting humans. Interventions are important, to protect the health of the animal as well as the consumer. Many established interventions involve chemical sanitisation agents (Doyle & Erickson, 2012) which are important in pathogen management but are not in the scope of this review. The biological approaches to meat safety and quality examined in this review are summarised in Table 1.

**Preharvest**

Biological systems are messy. In humans, the microbiota contains about 100 trillion bacteria from 500 to 1000 distinct species that provide multiple benefits to the host (Rohhion & Chassaing, 2016). A proportional number of bacteria could be expected in other animal systems. While this environment is ideal for beneficial and necessary microbial populations, it also can play host to enteric pathogens that may be able to outcompete the normal microbial flora, leading to infection or disease. A healthy microbial population is an organism’s first and best defence against such infections.

In the past, animals were routinely given a subtherapeutic level of antibiotics to promote growth, known as antibiotic growth promoters (AGP), in their feeding regimen. Unfortunately, long-term usage of AGP has the added risk of the emergence of drug-resistant microorganisms (Gaggia et al., 2010; Markowiak & Slizewska, 2018). The EU banned the use of AGP as of 1 January 2006 (Nawab et al., 2018). In 2013, the United States FDA released Guidance for Industry #213, which provides two recommended principles regarding the appropriate or judicious use of medically important antimicrobial drugs: (i) Limit medically important antimicrobial drugs to uses in animals that are considered necessary for assuring animal health, and (ii) Limit medically important antimicrobial drugs to uses in animals that include veterinary oversight or consultation (U.S. Food & Drug Administration, Dept of Health & Human Services, 2013). This reduction of AGP had predictable consequences: without the growth promoter, livestock and poultry did not grow as rapidly and pathogens became more prevalent. According to Niewold (2007), antimicrobial growth promoters enhance growth of animals by affecting intestinal physiology, inflammation, and immune response, thus also affecting changes in intestinal microbiota. Even with the use of AGP, pathogen load remains a significant problem, and the pathogens found often exhibited antibiotic resistance (Gaggia et al., 2010). In addition, the normal flora could be disrupted by the antibiotic therapy, destroying the healthy balance and making the livestock more susceptible to infection (Swaggerty et al., 2018). A replacement therapy was desirable for AGP, without the side effects of antibiotics. Some biological strategies developed for preharvest use include probiotics/prebiotics/synbiotics with related bacteriocins and competitive exclusion (CE), bacteriophage, and vaccination.

**Probiotics, prebiotics and synbiotics – direct-fed microbials**

One method of control of gut pathogens involves the utilisation of probiotic organisms. The idea of using probiotic organisms for their health benefits trace back to the observations of Elie Metchnikoff as reported in his book, The Prolongation of Life: Optimistic Studies, when he related the longevity of Bulgarian peasants with their consumption of yoghurt and the ‘Bulgarian bacillus’ (Metchnikoff, 1907). The definition most
Table 1 An overview of biological approaches to meat safety

| Stage of production | Biological method                  | Action                        | References                                                                 |
|---------------------|-----------------------------------|-------------------------------|---------------------------------------------------------------------------|
| Preharvest          | Direct-fed microbials (DFM)       | Growth enhancement            | Ruminants – Khan et al. (2016); McAllister et al. (2011); Oliver et al. (2009) and Timmerman et al. (2005) |
|                     | (Probiotic/prebiotic/symbiotic)   |                               | Non-Ruminants – Tufarelli et al. (2017)                                    |
|                     |                                   |                               | Poultry – Park et al. (2016); Mountzouris et al. (2007) and Khafsefidi & Rahimi (2005) |
|                     |                                   | Reduction of pathogen load    | Beef – Wisener et al. (2015) and Schamberger et al. (2004)                |
|                     |                                   |                               | Sheep – Rigobelo et al. (2014)                                            |
|                     |                                   |                               | Goats – Maragkoudakis et al. (2010)                                        |
|                     |                                   |                               | Poultry – Ebeid et al. (2019); Forkus et al. (2017) and Saint-Cyr et al. (2016) |
|                     |                                   | Competitive exclusion         | Beef – Swaggerty et al. (2018) and Brashears et al. (2003)                |
| Carcass handling    | Bacteriophage                     | Very specific targeting of bacteria | Swaggerty et al. (2018); Klopatek et al. (2018); Doyle & Erickson (2012); Wall et al. (2010); Callaway et al. (2008) and Sheng et al. (2006) |
|                     |                                   | Implantation                  | Swaggerty et al. (2018); Doyle & Erickson (2012) and Oliver et al. (2009) |
| Post-harvest        | Bacteriophage treatment           | Improvements not statistically significant | Arthur (2017) |
| processing          | Bacteriocins, colics, etc.        | Produced by bacteria; active against similar bacteria | da Costa et al. (2019) |
|                     |                                   |                               | In situ- Isa & Rasavi (2018); Hu et al. (2017); Swetwiwathana & Visessanguan (2015); Toderov et al. (2013) and Drosinos (2006) |
|                     |                                   |                               | Cello-free supernatant – Vijayakumar & Muriana (2017); Kamble et al. (2017); Rivas et al. (2017); Unlu et al. (2016) and Nielsen et al. (1990) |
|                     | Bdellovibrio bacteriovorus        | Parasitic bacteria predatory against other Gram (–) bacteria | Ottaviani et al. (2019) |
|                     | Bacteriophage                     | Precisely defined targets     | In meat products – Thung et al. (2019); Sirdesai et al. (2018); de Melo et al. (2018); Hagens et al. (2018); Gutierrez et al. (2017); Yeh et al. (2017); lacumin et al. (2016); Spricigo et al. (2013); Doyle & Erickson (2012) and Guenther et al. (2009) |
|                     |                                   |                               | On meat surfaces – Carter et al. (2012); Abuladze et al. (2008) and O’Flynn et al. (2004) |
|                     |                                   |                               | On hard surfaces – Ravensdale et al. (2018); lacumin et al. (2016); Guiterrez et al. (2016); Woolston et al. (2013) and Abuladze et al. (2008) |
|                     | Nitrite reduction                 | Acid-producing cultures enhancing nitrite effectiveness | Nikodinoska et al. (2019); Di Gioia et al. (2016); Li et al. (2013) and Tanaka et al. (1985a) |
|                     | Hurdle technology                | Using two or more preservation methods in combination for optimum food safety | Nikodinoska et al. (2019); Ramaroson et al. (2018); Wang et al. (2017); Sukumar et al. (2015); Alahakoon et al. (2015); Carter et al. (2012); Garriga et al. (2002) and Hugas (1998) |

commonly used for probiotics today, ‘live organisms which when administered in adequate amounts confer a health benefit to the host’, (Hill et al., 2014) may not be broad enough. The Office of Regulatory Affairs of the Food and Drug Administration (FDA) of the United States, as well as the Association of American Feed Control Officials, has guided the term ‘direct-fed microbial’ (DFM) be used instead of ‘probiotic’ to describe feed products that contain a source of live naturally occurring microorganisms (Brashears et al., 2020 The Authors. International Journal of Food Science & Technology published by John Wiley & Sons Ltd © 2020)
probiotics has, in addition, been shown to significantly reduce the load of food-borne pathogenic microorganisms. A recent study showed that the dietary inclusion of *Bacillus subtilis* as a probiotic in broiler chicken significantly reduced the counts of *Escherichia coli* and *Salmonella* both in the gut and in the litter (Ebeid et al., 2019). Another poultry study showed a 97% reduction of *Salmonella enteritidis* in the ceca of turkeys treated with an engineered probiotic *E. coli* (Forkus et al., 2017). Campylobacter colonisation in broiler chickens has been shown to be limited easily and inexpensively by the oral administration of probiotic LAB, especially using *Lactobacillus* spp., such as *acidophilus*, *casei*, *crispatus*, *gasseri*, *helveticus*, *pentosus*, *plantarum*, *rhamnosus* and *salivarius* (Saint-Cyr et al., 2016). A review of literature in 2015 concluded that feeding beef cattle DFM during the growing period was efficacious in reducing faecal shedding of *E. coli* 0157:H7, with the caveat that the research considered was performed in feedlots with a low stocking density (Wisener et al., 2015). Adding certain colicinogenic *E. coli* strains to the feed reduced faecal shedding of *E. coli* 0157:H7 in calves (Schamberger et al., 2004). A probiotic preparation containing *L. acidophilus*, *L. helveticus*, *L. bulgaricus*, *L. lactis*, *S. thermophilus* and *E. faecium* significantly reduced faecal shedding of Shiga toxin-producing *Escherichia coli* (STEC) in sheep, demonstrating its possible potential as a preharvest intervention method aimed at reducing food-borne illness in humans (Rigobello et al., 2014). A promising *L. plantarum* isolate was used as a feed supplement in lactating goats resulted in a significant increase in LAB coupled with a significant decrease in faecal clostridia (Maragkoudakis et al., 2010).

One way that probiotic treatment in preharvest animals contributes to meat safety is through competitive exclusion (CE), defined as the response of healthy gut microbiota to protect the intestine from the establishment of pathogens, reducing infection of the gastrointestinal tract in animals (Nawab et al., 2018). The goal of CE cultures is the exclusion of pathogens from the gut of a neonatal host or the displacement of established pathogens in older hosts (Nurmi et al., 1992). This technique can be administered in two ways. The classic form of CE, known as the ‘Nurmi concept’ after its author, was developed in the 1970s as a safeguard against *Salmonella* colonisation in poultry and involved dousing newly hatched chicks with a suspension of gut content prepared from healthy adult chickens (Nurmi & Rantala, 1973). This introduction of CE bacteria should occur early in life to allow the establishment of the beneficial bacteria in the gastrointestinal system, thus protecting the bird from opportunistic pathogens through competition or antagonism (Gaggia et al., 2010). It is assumed that this approach works because the bacteria preparation administered to the

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Bacteriophages

Bacteriophages (phages) are viruses that infect bacteria and reproduce within them. There are two groups of phage: lysogenic or temperate, where the viral genetic material integrates into the host DNA and is replicated with the host cell DNA, and lytic, where the phage infect their target host, reproduce, then lyse the bacterial cell, releasing their daughter phage (Doyle & Erickson, 2012). It is vital that only strictly lytic phage, with no transfer of genetic material, is used because phage-mediated transfer could have dangerous unintended consequences. Phage-mediated transfer is believed to be the mechanism by which STEC acquired their Shiga toxin genes (Swaggerty et al., 2018). Phage has precisely defined targets that limit the effectiveness of the phage to very specific bacteria, down to the species or even serotype. They are usually presented in a cocktail of several phages using differing receptors and host bacterial strains to maximise effectiveness and minimise resistance. The ideal treatment would be with either a bacteriophage targeting common surface receptors of several pathogens or else a mixture of several phages, each targeting a specific receptor of one or more pathogen subtypes (Doyle & Erickson, 2012).

Delivery of the phage to a site in the host animal where it can be effective has been a challenge. Monogastric animals (swine, poultry) have a highly acidic stomach that serves as a barrier to microbial passage, though some microorganisms do get through. These harsh conditions mean that phages administered orally must be protected from degradation or be highly acid resistant (Doyle & Erickson, 2012; Klopatek et al., 2018). Poultry has an additional complication in having a pre-gastric crop, subject to colonisation by Salmonella and Campylobacter (Klopatek et al., 2018). Though not as harsh, the complicated digestive system of ruminants provides its own challenges. Buffering agents or microencapsulation are two possible solutions in use to protect phage from the harsh environments of the digestive tract (Doyle & Erickson, 2012). While Salmonella and Campylobacter can be found throughout the digestive tracts of ruminants, the primary site of colonisation of EHEC is the recto-anal junction. Sheng et al. (2006) devised a successful strategy of applying previously characterised 0157-specific bacteriophage directly to the rectal-anal mucosa of steers. This direct application, in combination with phage in the drinking water, significantly reduced, but did not eliminate E. coli 0157:H7. Another study examined the oral inoculation of a phage cocktail in experimentally infected sheep (Callaway et al., 2008). Again, the model showed reduction, but not elimination. A microencapsulated phage cocktail prep administered by oral gavage to non-infected pigs reduced Salmonella colonisation in a Salmonella-contaminated newly hatched chicks competitively excludes Salmonella from sites that the pathogen would occupy if no other bacteria were present. The mechanisms by which this is accomplished may include any or all of the following: effects on the immune system, interference with adhesion, competition for nutrients or production of inhibitory molecule. The CE with undefined cultures of porcine origin plus B. thetaiotaomicron was also tested on pigs (Joerg & Ganguly, 2017). The CE using defined cultures seems to be the method of choice as a preharvest intervention strategy against E. coli 0157:H7 in cattle (Swaggerty et al., 2018). Cattle are currently considered to be the primary source of Enterohemorrhagic E. coli (EHEC) such as E. coli O157:H7 in the food supply. It appears to be widespread among cattle herds in the United States, though individual animal prevalence is low and transient. Brashears et al. (2003) tested numerous LAB isolates to determine their effect on E. coli 0157:H7 and found positive results as LAB single strains or in combination were capable of reducing E. coli 0157:H7 shedding and carcass contamination.

Many bacteria directly inhibit intestinal pathogens by competing for nutrients, inducing the starvation of competing pathogens (Rolhion & Chassaing, 2016). Others inhibit pathogenic strains by the production of bacteriocins, antimicrobial peptides with activity to selectively kill or inhibit the growth of competing bacteria. These bacteria may be introduced to the host animal as DFMs. While bacteriocins produced by LAB are generally only effective in inhibiting the growth of Gram-positive organisms, some strains of E. coli produce bacteriocins (colicins and microcins) shown to inhibit Gram-negative pathogenic microorganisms like Salmonella and enterohemorrhagic E. coli (Tkalcic et al., 2003; Gillor et al., 2004; Rolhion & Chassaing, 2016). Administration of colicin and microcin producing bacteria may be effective in reducing enteric pathogenic bacteria levels in the host animal gut and may even help prevent initial infection (Gillor et al., 2004).

An interesting parasitic bacterium, Bdellovibrio bacteriovorus, that preys on Gram-negative bacteria, including E. coli, has been studied for many decades. Not capable of infecting eukaryotic cells and not harmful to Gram-positive bacteria, fungal or yeast starter cultures, but part of the normal flora of healthy humans, B. bacteriovorus could be utilised to reduce both pathogenic and spoilage organisms in food. Spread on chicken slices, the E. coli prey reduction was 4.3 log when compared with control at 6 h (Ottau viani et al., 2019). This bacterium, and other Bdellovibrio and like organisms (BALOs) show promise against a large variety of Gram-negative pathogens and could be useful in multiple applications to improve safety and shelf life of meat products.
holding pen (Wall et al., 2010). The use of phage therapy in poultry yielded similar results reducing but not eliminating Salmonella and Campylobacter in the flocks (Klopatek et al., 2018). Bacteriophage therapy does significantly reduce pathogen carriage in host animals, but based on the transient nature of the therapy and the possibility of development of phage resistance, phage therapy should be administered just before harvest, preferably to ‘super shedders’ to minimise contamination (Doyle & Erickson, 2012).

Immunisations

Another biological approach to meat safety is employing the animal’s own immune system against the foodborne pathogen via vaccination. As in human vaccines, the vaccines utilise either live or attenuated bacteria to promote an immune response. Live vaccines generally offer better protection against pathogens; however, some public health decision makers are concerned about their safety in the food production line, especially when genetic manipulation has been used in its production (Doyle & Erickson, 2012). Traditionally, vaccines were mostly developed against bacteria or viruses that affected the health of the animal host but vaccines have been developed that are effective against Salmonella and E. coli in swine and cattle as well as Salmonella in poultry (Oliver et al., 2009). Despite being commercially available, vaccines against Shiga toxin-producing E. coli (such as serotype 0157:H7) are not in wide usage. The vaccine is expensive to implement, and the market does not yet compensate the producer for this added cost (Swaggerty et al., 2018). Another limitation of vaccination is that immunised animals often produce antibodies against the vaccine strain, making it difficult to differentiate using serological testing between vaccinated animals and infected ones (Doyle & Erickson, 2012). And, as in so many other interventions in the preharvest animal, vaccination reduces, but does not clear the pathogen from the host and should be considered as another step in a comprehensive program aimed at reducing the pathogen load in the production of meat (Swaggerty et al., 2018).

Post-harvest

Carcass care

Food safety practices continue after the animal is harvested. In order for meat products to be sold commercially in the United States, they must be inspected and passed by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS). Inspection is required by the Federal Meat Inspection Act (FMIA) and is completed by the USDA-FSIS. Federally inspected meat companies are required to have and follow Sanitation Standard Operating Procedures (SSOP) and HACCP plans. A USDA-FSIS inspector must be present at all times during harvest and at least part of the day when further processing is occurring. Inspectors are responsible for verifying humane handling, conducting ante-mortem and post-mortem inspections of the animals and carcasses to ensure the meat is fit for human consumption, ensuring sanitary conditions, and reviewing records to ensure accurate documentation (SSOPs and HACCP) and compliance with requirements. The FMIA was developed to help ensure a safe, wholesome and properly labelled product. However, in order to ensure every food product was safe to consume, you would have to test every part of every product which would not leave any food for consumption. This section will address post-harvest challenges and potential strategies to address these challenges.

Cattle hides are major sources of carcass contamination during beef processing (Bosilevac et al., 2005). Animals are subject to surface contamination with manure throughout life, including at the farm and during transportation. Attempts are being made to reduce stresses to the animal during transport and holding as stressed animals are more likely to spread pathogens such as Salmonella or E. coli to other animals and/or become infected themselves. Since beef carcasses from highly contaminated animals have been shown to retain a higher microbial load than cleaner animals, preslaughter washes may be an effective strategy (Kotula & Kotula, 2000). Packing plants require a zero-tolerance policy; in which before carcasses are rinsed and allowed to enter the cooler there must be no visible contamination including faecal material, hair or mammary fluid. Bacteriophage therapy has been proposed as a preharvest microbial intervention to reduce the incidence of E. coli 0157:H7 on cattle hides (O’Flynn et al., 2004), but in an experiment conducted by Arthur et al. (2017), using a phage spray treatment applied in the holding pen area of a commercial beef processing plant, the improvements were not statistically significant. Currently, the most effective carcass decontamination techniques are not biological. washes involving chemical agents, acids, steam and hot water (Bosilevac et al., 2005; Sevart et al., 2016) have been shown effective. Another non-biological procedure involving treatment with near-infrared spectroscopy has also been examined (Chapman et al., 2019), but neither of these approaches are in the scope of this review.

Packing plants incorporate a test and hold policy as part of their HACCP plan in which every combo bin is representatively sampled for any pathogenic microorganisms that make it through the harvest and carcass fabrication process. Meat combo bins that
come back with a positive test are either discarded or utilised in a fully cooked product produced by the company to ensure the product reaches a temperature to kill the pathogen. Care must be taken to not contaminate the meat through all subsequent processing steps.

Biopreservation systems have been gaining attention as a means of ‘naturally’ controlling the growth of pathogenic and spoilage organisms in ready-to-eat (RTE) foods (Unlu et al., 2016). Biopreservation is defined as ‘the use of LAB and/or their metabolites to improve the safety and quality of foods that are not fermented’ (Matthews et al., 2017). Biopreservation techniques we discuss here include bacteriocins (produced in situ or added), organic acids and bacteriophage. We also examine the use of protective cultures in reducing the need for nitrates, look at combination preservation methodologies (i.e. hurdle technology) and fermentation, which naturally employs such technology.

**Bacteriocins**

Bacteriocins are small ribosomally synthesised antimicrobial compounds produced by LAB and other bacteria (O’Sullivan et al., 2002). While the bacteriocins produced by these bacteria can kill or inhibit phylogenetically related species, or sometimes totally unrelated microorganisms, they do not affect the producing microorganism. Ross et al. (1999) generalises that the potential applications of specific bacteriocins can be predicted by their properties, with characteristics such as host range, pH and heat stability being the most important. The use of bacteriocins in meat biopreservation systems is an additional safety measure for minimally processed products (da Costa et al., 2019) and may also help meet the consumer demand for natural preservative techniques.

Bacteriocins can be either produced in situ by inoculating meat products with bacteriocin-producing protective or starter cultures or they can be harvested from in vitro culturing techniques and applied as purified or partially purified bacteriocin food additives. In situ bacteriocin production is the most cost-effective, especially for fermented meat products but may not always be suitable. The direct addition of bacteriocin preparations is more appropriate when live LAB are unable to produce bacteriocins in real meat systems (Woraprayote et al., 2016). And sometimes, when neither of these methods work, the bacteriocin can be effectively incorporated into the packaging.

Many of the most promising bacteriocins have been isolated from the very food items in need of protection. The bacterium *L. alimentarius* that produced a novel bacteriocin, lactocin MM₄₄, was isolated from a traditional Chinese meat product (Hu et al., 2017). A bacteriocin strain of the probiotic organism *P. pentosaceus* was incorporated into a starter culture for a traditional Thai fermented meat, and its bacteriocin, pediocin PA-1, was found to be inhibitory against *S. anatum* (Swettiwathanha & Visessanguan, 2015). Three *L. sakei* spp. isolated from a traditional sausage from Portugal were found to produce high levels of bacteriocins effective against several food-borne pathogens, indicating their potential for use in a mixed starter culture (Toderov et al., 2013). Another study found a sakicin P-producing *L. sakei* well suited for growth in complex meat batter environments for the production of several European fermented sausages, significantly reducing *L. monocytogenes* contamination. Their experiments adding the purified sakicin P were less successful, likely due to low solubility, uneven distribution, lack of stability, and absorption of the bacteriocin to fats and other meat components of the batter (Drosinos et al., 2006). Lactobacillus acidophilus showed a significant biopreservative effect against *L. innocua* in minced meat and was even more effective combined with *Bifidobacterium animalis* BB12. (Isa & Rasavi, 2018).

Semi-purified cell-free supernatants (CFS) were applied to meat products with good results. Unlu et al. (2016) produced freeze-dried bacteriocin-containing powders with which hot dogs were treated and showed a >2-log reduction of a listerial cocktail. Vijayakumar & Muriana (2017) performed a similar experiment, dipping hot dogs into a bacteriocin mixture based on mode of action and had similar results of >2 log reduction of *Listeria monocytogenes* 39-2. Nielsen et al. (1990) dipped fresh beef slices into a liquid CFS from *Pediococcus acidilactici* showing an inhibitory effect on *L. monocytogenes*. Kamble et al. (2017) sprayed chicken carcases with pediocin NCDC252 CFS and showed a reduction in total viable count with improved appearance and odour over controls over 6 days’ time. Another recent study infused casings by dipping them into CFS containing Sakicin G before filling with a sterile meat emulsion and surface challenging with *L. innocua*. All treated casings had a bacteriostatic effect, except collagen, which seemed to inhibit listerial growth (Rivas et al., 2017).

An interesting novel study found broad and efficient control of enterohemorrhagic *E. coli* using plant-produced colicins (Schultz et al., 2015). This study showed that plant-made recombinant proteins can provide relatively large amounts of a variety of colicin types active against *E. coli* 0157:H7, making colicins a viable solution to reduce food-borne pathogenic bacteria entering the food supply (Calloway & Sheridan, 2015).

Though a wide range of these inhibitors have been characterised, at this time only nisin has been approved by the FDA for use as a food preservative.
Bacteriophage

Lytic bacteriophages are useful in the control of foodborne pathogens in meat products. Their special characteristics, particularly their target specificity, rapid bacterial killing and ability to self-replicate within their specific host organism make them especially appropriate in food protection applications (Spricigo et al., 2013). As in the preharvest animal, the phage employed has precisely defined targets that limit the effectiveness of the phage to specific bacteria, making phage use safe in foods for human consumption, as the phage would not affect the normal gut microbiota of human as antibiotics would. They are usually presented in a cocktail of several phages using differing receptors and host bacterial strains to maximise effectiveness and minimise resistance. Again, the ideal treatment would be with either a bacteriophage targeting common surface receptors of several pathogens or a mixture of several phages, each targeting a specific receptor of one or more pathogen subtypes (Doyle & Erickson, 2012).

Phage isolates have been found to be effective in treating Listeria-contaminated meat products. Virulent (lytic) broad-host bacteriophages A511 and P100 were evaluated against two strains of L. monocytogenes contaminating ready-to-eat foods including hot dogs and cold cuts, and both were found to be effective (Guenther et al., 2009). Phage P100 was also found to be effective against Listeria contamination on slices of dry-cured ham (Iacumin et al., 2016; Gutierrez et al., 2017). Listeria phage P100 has GRAS status for food applications (U.S. Food & Drug Administration, 2007). The first phage cocktail, ListShield™, was approved in 2006 by the FDA leading to many more phage preparations available commercially for pathogen control. Significant reductions of pathogenic bacteria have been observed in artificially contaminated food products treated with these commercial phage cocktails (de Melo et al., 2018).

Salmonella contamination has also been addressed in phage research. A phage cocktail used on experimentally contaminated pigskin and chicken breast resulted in significant bacterial reductions (Spricigo et al., 2013). Another study employed a commercial bacteriophage product Salmonelex™ on contaminated red meat trim and poultry during tumbling, providing additional control of Salmonella in ground products (Yeh et al., 2017). Two additional studies involving the use of another commercial anti-Salmonella phage product, PhageGuard S, showed significant Salmonella reduction in pork (Sirdesai et al., 2018) and poultry products (Hagens et al., 2018). Thung et al. (2019) isolated a lytic bacteriophage from retail meat samples, then successfully treated Salmonella-contaminated sliced beef and chicken obtaining a 2-log reduction.

A study of a three-bacteriophage cocktail selected against E. coli 0157:H7 in an initial meat trial experiment found that E. coli was completely eliminated from the meat surface in seven of nine cases (O’Flynn et al., 2004). Another study, employing the commercial three-phage cocktail EcoShield™, significantly reduced the bacterial load of experimentally contaminated beef by 94%, but did not protect the product from recontamination (Carter et al., 2012). Abuladze et al. (2008) treated ground beef samples with a three-phage cocktail, reducing the contamination level by 95%.

Bacteriophage has also been shown effective in reducing contamination of hard surfaces. Woolston et al. (2013) showed a rapid reduction of susceptible Salmonella spp. on glass and stainless-steel surfaces when treated with a commercial 6-phage cocktail. It was totally ineffective against other Salmonella spp., underscoring the fact that phages are very specific, and the correct ones must be included. When two-component phages were replaced with ones targeting the missed Salmonella, the new cocktail became effective against that strain, showing that phage preparations can be effectively customised for the required application (Woolston et al., 2013). Abuladze et al. (2008) treated glass coverslips and gypsum boards with a three-phage cocktail targeted against E. coli 0157:H7, achieving significant reductions. Phage treatment may be a valuable tool in preventing food contamination from surfaces during processing.

Bacterial biofilm, complex communities of bacteria protected by an extracellular polymeric material, is a persistent source of microbial contamination in processing plants. This matrix keeps the bacteria in close proximity to each other, leading to a complex symbiosis in water, nutrient, oxygen and enzyme distribution and providing a microenvironment for the thriving and the mutual protection of involved bacterial species (Gutierrez et al., 2016). The biofilm makes the industrial surfaces resistant to normal cleaning and disinfection processes, leading to contamination of meat.
during processing and packaging. Iacumin et al. (2016) found that a suspension of anti-listerial phage eliminated free L. monocytogenes cells and biofilm. Commercial phage-based products ListShieldTM and ListexTMP100 were effective in removing several listerial biofilms on stainless-steel or polystyrene surfaces (Gutierrez et al., 2017). Ravensdale et al. (2018) related one potential effect of phage treatment against biofilm was through the disruption of quorum sensing, preventing the cells to coordinate processes and regulation.

Nitrite reduction – protective cultures

Sodium or potassium nitrite has been used in the curing process of meats for several beneficial reasons: nitrites react with myoglobin to form the characteristic pink colour of cured meats, contribute to the cured meat flavour and texture, delay oxidative rancidity, and inhibit bacterial growth, notably C. botulinum among other bacterial pathogens (Li et al., 2013). Unfortunately, nitrites could react with amines in meat protein at high temperatures, forming carcinogenic nitrosamines (Di Gioia et al., 2016). This is one of many reasons that an increasing number of consumers demand natural chemical-free products.

Following a USDA initiative aimed at reducing nitrites in bacon, researchers at the University of Wisconsin-Madison developed a novel approach to curing. This ‘Wisconsin Process’ involved reducing levels of nitrite and adding a readily fermentable carbohydrate (sucrose) and an acid-producing culture (Pediococcus acidilactici) (Tanaka et al., 1985a; Gould, 2000). The acid-producing culture can ferment the carbohydrate, reducing the pH and enhancing the effectiveness of the nitrite (Gould, 2000). Tanaka et al. (1985a) found that under these conditions the test bacon had substantially better anti-botulinal properties than the control bacon. Residual nitrite levels were lower and N-nitropyridine levels far lower. As important, the sensory aspects of the bacon did not suffer, with all samples liked equally well (Tanaka et al., 1985b).

Ongoing research in meat models has identified other LAB strains that appear to be effective in pathogen control in a reduced nitrite environment. L. plantarum PCS20, a bacteriocin-producing strain, was effective against Clostridium (Di Gioia et al., 2016) and against Listeria, but not Salmonella (Nikodinoska et al., 2019), in ground pork. Another study found that Staphylococcus xylosus was able to convert metmyoglobin into nitrosomyoglobin in raw pork batters, imparting the pink colour of cured meats without the addition of nitrite, demonstrating a potential solution for colouring cured meat products without adding nitrite (Li et al., 2013). Alternative curing methods have been developed utilising vegetable sources of nitrate coupled with nitrate-reducing bacteria to facilitate in situ generation of nitrite, producing the identical nitrosyl haem pigment achieved with traditional nitrite curing (Sindelar & Milkowski, 2012).

Hurdle technology

The use of two or more methods in combination with optimise food preservation is known as hurdle technology. The goal is to increase food safety, while minimising sensory qualities of the food product. While many of these ‘hurdles’ involve manipulating water activity, pH, temperature, redox potential, among others, biological control methods are also welcome hurdles in assuring a safe food supply. Hurdle technology also helps in the fight against the development of resistant strains against any one hurdle. The inclusion of LAB, bacteriocins and/or bacteriophages have been shown to be an effective hurdle in combination with added chemicals such as lauric arginate (LAE), cetlypyridinium chloride (CPC), chlorine, peracetic acid (Sukumar et al., 2015), potassium sorbate (Nikodinoska et al., 2019), sodium nitrite (Wang et al., 2017) or high pressure or pulsed field processing (Hugas, 1998; Garriga et al., 2002; Ramaroson et al., 2018). One study found that heat treatment makes Clostridium 10-fold more sensitive to the bacteriocin nisin (Carter et al., 2012; Alahakoon et al., 2015).

Lactic acid fermentation has been utilised to preserve meats for thousands of years. LAB may be selected as starter culture and inoculated into the meat, or naturally occurring LAB are encouraged to ferment available carbon sources, while other bacteria are discouraged through methods such as salting. Organic acids and other metabolic products are produced in this process, further inhibiting pathogenic bacterial growth. Fermentation methods naturally combine several hurdles (reduced pH, reduced water activity (a_w), bacteriocins, organic acids, etc.) to achieve a safer meat product. Adding further hurdles, such as temperature controls, could increase safety.

Conclusion

Biological interventions can be successfully used to increase meat safety, both in preharvest and meat processing conditions. The use of probiotics and prebiotics as direct-fed microbials can result in a healthy animal less prone to colonisation by human pathogenic bacteria, and thus less prone to contaminate the food stream. Vaccination and phage therapies can also be effective in keeping meat safe. While phage treatments have been shown to be effective in both live animals and in meat processing, phage washes do not appear to be effective as a hide wash against E. coli.
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0157:H7. More study into this use of phage therapy may be appropriate.

In the processing of meat products, hurdle technology using biological steps has been shown to be effective. LAB fermentation is an ancient procedure that promotes meat safety through the formation of organic acids causing a drop in pH and through the in situ production of bacteriocins. Where fermentation is not appropriate, bacteriocins derived from LAB could be a viable solution. Alternatively, a bacteriophage specific to the target food-borne pathogen could be employed. All of these biological approaches to food safety are more effective when used in concert with other hurdles for the safest minimally processed meat possible for the consumer. Biological approaches will become increasingly more popular in meat safety and quality procedures as techniques are developed with good safety records.

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Conflict of interest

There are no conflicts of interest in this work by any of the authors.

Ethical approval

Ethical approval was not required for this research.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Author contribution

Barbara Nielsen: Conceptualization (Equal), Project administration (Equal), Writing-original draft (Lead), Writing-review & editing (Equal). Michael J. Colle: Conceptualization (Equal), Project administration (Equal), Writing-original draft (Equal), Writing-review & editing (Equal). Gülhan Unlu: Conceptualization (Lead), Project administration (Lead), Writing-original draft (Equal), Writing-review & editing (Equal).

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