MICROBES AS A TOOL TO DEFEND AGAINST ANTIBIOTIC RESISTANCE IN FOOD ANIMAL PRODUCTION

A. K. BHUNIA

Molecular Food Microbiology Laboratory

Department of Food Science

Department of Comparative Pathobiology

Purdue Institute of Inflammation Immunology and Infectious Diseases

Purdue University, West Lafayette, Indiana 47907, USA

The antibiotic-resistant microbial community collectively referred to as antibiotic resistome is on the rise and largely attributed to indiscriminate use or misuse of antibiotics in food animal production, human medicine and in agriculture. Scientists are constantly looking for an answer to provide an effective and safe alternative to overcome the growing problem of antibiotic resistance. The best practice would be to reduce antibiotics use; however, it may not be adequate. Antimicrobials are necessary for food animal production, and treatment of diseases; therefore, alternative strategies must be employed. Plant or animal-derived antimicrobials, organic acids, prebiotics, immunostimulants, and new generation eco-friendly antibiotics have been proposed. Are they effective and credible to lessen our global antibiotic resistance crisis? In recent years, however, the use of microbes as a tool to defend against antibiotic resistome is gathering momentum. These microbes include bacteriophages, undefined or defined mixed microbial cultures, probiotic bacteria, and bioengineered microbes which may be able to reduce our dependence on antibiotics in food animal production and alleviate antibiotic resistance crisis.

Key words: Antibiotic resistance, Bacteriophages, Bioengineered, Microbiota, Probiotic

Evolution, adaptation and natural selection have shaped all living creatures, small or large on this planet. In all living entities, exposure to life-threatening conditions can trigger a coping mechanism. Microscopic organisms are also frequently being exposed to an array of antimicrobials including ultraviolet light, acids, alkali, chemical sanitizers, antimicrobial peptides, and antibiotics. Repeated exposure works as a selective pressure, which prompts microorganisms to undergo genetic mutation, or up-regulation or down-regulation of certain

genes to develop a robust defense against a threat. Another interesting evolutionary aspect is that antibiotics disrupt microbial ecosystem thus enabling persistence of resistant population. Therefore, all microbes, irrespective of being primary pathogens, opportunistic pathogens or commensals have an equal chance of being exposed to the same antimicrobials when applied to food animals for growth promotion or therapeutic application. Therefore, they can contribute to the expansion of antibiotic resistome in the

environment, animals, and humans. It has been suggested that antibiotic resistance for animal-specific pathogens may be a concern for veterinary medicine but may not be a major concern for human medicine. However, any veterinary drugs that are responsible for the induction of antimicrobial resistance (AMR) in zoonotic pathogens and commensals can have a direct impact on human health (Toutain *et al.*, 2016).

Since the discovery of penicillin in 1928 by Alexander Fleming and its introduction as therapeutics in the 1940s, our confidence in combating disease-causing microorganisms grew exponentially without ever discerning of its long-term consequences. Now it has been well recognized by the scientific community that extensive and indiscriminate use of antibiotics in food animals, aquaculture and poultry for human food production and as therapeutics for treatment of human and animal diseases have fueled the crisis of antibiotic resistance (Neu, 1992; Davies and Davies, 2010; Beceiro *et al.*, 2013; Rodriguez-Rojas *et al.*, 2013; Van Boeckel *et al.*, 2015; Pérez-Rodríguez and Mercanoglu, 2019). The most important factors attributed to the onset of AMR is when antibiotics are used at a sub-inhibitory or sublethal dosage and such nonlethal exposure may cause evolution and selection of antibiotic-resistant bacteria (Gullberg *et al.*, 2011; Andersson and Hughes, 2014; Zhu *et al.*, 2018) including the emergence of multidrug-resistant (MDR) bacterial pathogens, such as extended-spectrum β -lactamase (ESBL) producing Gram-negative bacteria (Carlet *et al.*, 2012; Blair *et al.*, 2014). However, discovering antibiotic resistance genes from ancient soil samples certainly, challenge our perception of the modern AMR

problem. Metagenomics analysis of ancient soil samples such as 40,000-year-old permafrost suggests the presence of genes encoding resistance to β -lactam, tetracycline and glycopeptide antibiotics (D'Costa *et al.*, 2011).

Nevertheless, antibiotic resistance is now widespread among different bacterial pathogens and even to different classes of antibiotics that target various cellular components, i.e., cell wall, protein, DNA, RNA, folic acid and mycolic acid synthesis. It has been estimated that about 80% of all antibiotics sold in the United States have been used in food animals as a growth promoter or to treat an animal infection (Marshall and Levy, 2011). Antibiotic resistance has been considered a global public health crisis and it has been predicted to cause 10 million deaths per year by 2050 (O'Neill, 2014). Though these numbers have been argued to be an overestimate (de Kraker *et al.*, 2016), the scientists agree that AMR problem is real and the situation would be grave unless remedial actions are taken (Toutain *et al.*, 2016; Ferri *et al.*, 2017; Pérez-Rodríguez and Mercanoglu 2019). In the U.S., antibiotic-resistant bacteria have been responsible for more than 2 million infections and 23,000 deaths annually (CDC, 2018) while in the European Union (EU), it is about 672,000 infections and 33,000 deaths per year (Cassini *et al.*, 2019).

The scientific community is constantly looking for an answer to provide an effective and safe alternative to antibiotics to overcome the growing problem of antibiotic resistance. In order to develop strategies to combat antibiotic resistance one has to recognize how wide-spread this problem is and the potential sources of antibiotic resistance and the

contributing factors. It is nearly impossible to completely eliminate antibiotic resistance in microbes, but the clinical or animal husbandry practices that can reduce further development of acquired resistance to antibiotics should be explored. One caveat, any credible solution to reduce antibiotic resistance in bacteria should be able to withstand the resistant mechanisms used by bacteria in the first place. Therefore, in recent years, the use of microbes such as bacteriophages, undefined or defined mixed microbial cultures, probiotic bacteria, and bioengineered microbes as a tool to defend against antibiotic resistance has been proposed (Kutateladze and Adamia, 2010; Amalaradjou and Bhunia, 2013; Pamer, 2016; Ryan and Bhunia, 2017; Wong and Santiago, 2017; Relman and Lipsitch, 2018) and this review will examine benefits and limitations of such approaches.

Sources of antibiotic resistance

Antibiotics as a growth promoter in animal husbandry: In modern food animal production practices, animals are housed in large groups with high animal densities thus there is a chance for rapid disease spread. Antibiotics as therapeutic or prophylactics are of great importance in such farming practices (Wegener, 2003). Antibiotics are used in feed or water for the treatment of sick animals as therapeutic. Likewise, prophylactic use of antibiotics in feed is a common practice to prevent diseases caused by infectious agents (Wegener, 2003). In addition, antibiotics are also used for growth promotion and such antibiotic use is speculated to grow (Van Boeckel *et al.*, 2015). Antibiotic concentration is much higher for therapeutic and prophylactic applications while growth-promoting antibiotics are used at a dosage

that is sub-therapeutic, which may be considered sub-minimum inhibitory concentration (MIC) and is a major contributor for the development of human resistance. For example, increased incidence of vancomycin (glycopeptide antibiotic) resistant enterococci (VRE) in food animals and subsequently in humans was attributed to the use of avoparcin, a glycopeptide antibiotic in animal feed as a growth promoter (Bager *et al.*, 1997). The ban of avoparcin in animal feed in the EU has markedly reduced human VRE resistance (Wegener, 2003). Likewise, there is a concerted effort to prohibit the use of human therapeutic antibiotics such as penicillin, tetracycline, etc as growth promoters (Mathew *et al.*, 2007; Cheng *et al.*, 2014). Since 2017, the US Food and Drug Administration (FDA) has banned the use of “medically important antibiotics” as antibiotic growth promoters in food animals (Brüssow, 2017). To further alleviate concerns for the contribution of veterinary drugs for the onset of human resistance, there is an impetus for the development of new eco-friendly drugs that would have a minimal ecological impact on animal commensal and the environmental microbiome (Toutain *et al.*, 2016).

Antibiotics as a therapeutic drug:

Therapeutic application of antimicrobials in the treatment of specific pathogens in veterinary medicine has not been considered a major concern for the onset of antibiotic resistance for human health (Toutain *et al.*, 2016). However, the effect of veterinary antibiotics on the zoonotic pathogens and the commensal resident bacteria in the host can have serious consequences which can contribute to the abundance of antibiotic resistance in humans and in the environment. Increased incidence of antibiotic-resistant

foodborne *Salmonella*, *Campylobacter* and *E. coli* have been attributed to the use of antibiotics in food animals (Toutain *et al.*, 2016). Furthermore, the indiscriminate prescription of antibiotics in both human and veterinary medicine practices without definitive identification of a causative infectious agent can exacerbate the AMR problem. Therefore, the stewardship for antibiotic use in human or veterinary medicine practices should be greatly emphasized.

In addition, the application of sub-inhibitory or sublethal antibiotic dosage has been attributed to be a major contributing factor for the onset of antibiotic resistance. Sublethal exposure can occur not only from improper use of antibiotics during therapeutic application but also from patients (human/animal) fecal waste and also pharmaceutical industry waste. Both can contribute to the environmental sub-inhibitory antibiotic pool to serve as a major selection pressure for the development of antibiotic resistome (Bengtsson-Palme and Larsson, 2016).

To further alleviate concerns for the contribution of veterinary drugs for the onset of human resistome, the FDA has approved several veterinary antibiotics, referred to as Veterinary Feed Directive (VFD) drugs for the treatment of infectious diseases in food animals (Allen and Stanton, 2014). Examples of these antibiotics include tilmicosin, florfenicol, avilamycin, apramycin, hygromycin and many more, which are to be used only for a specified time period depending on the animal species (Allen and Stanton, 2014). However, a recent *in vitro* study showed that certain zoonotic pathogens (*Salmonella enterica*, *Listeria monocytogenes*

and *Staphylococcus aureus*) exposed to tilmicosin and florfenicol at a sub-inhibitory dosage exhibited acquired cross-resistance against several human antibiotics including ampicillin, tetracycline and nalidixic acid (Singh and Bhunia, 2019). This suggests that veterinary antimicrobials have the potential to induce cross-resistance in zoonotic pathogens thus would contribute to the increase in human antibiotic resistome.

Environment and wildlife: The environment has been considered a major source of antibiotic resistome (Ishii, 2019). Soil and aquatic environments are routinely exposed to animal and human waste. The residual antimicrobials (not metabolized) used in therapeutic or health-promoting applications can contaminate our environment through feces/urine. Water, soil, and wildlife including migratory birds can spread antibiotic resistance genes in the environment (Baquero *et al.*, 2008; Allen *et al.*, 2010; Bonnedahl and Järhult, 2014). Wastewater treatment plants are considered a hotspot for antibiotic resistance genes (Guo *et al.*, 2017). Furthermore, improper disposal of antibiotics in the hospital and clinics can be a major environmental source of antibiotics (Wright, 2010). Veterinary antibiotics have been found in aquatic and terrestrial environments (Kemper, 2008). Management of antibiotic resistance in the environment would be a crucial strategy to reduce the spread of AMR (Berendonk *et al.*, 2015).

Soil is a major source of antibiotic-resistant bacteria. Evidence suggests that the soil sample collected in the 1940s, showed an increased abundance of antibiotic-resistant genes (Knapp *et al.*, 2009). The evidence further shows shared antibiotic resistance in

soil bacteria and human pathogens (Forsberg *et al.*, 2012). Manure applications in the agriculture field can also aid in the increased growth of antibiotic-resistant bacteria in the soil (Udikovic-Kolic *et al.*, 2014).

Wildlife including migratory birds can spread antibiotic resistance genes very rapidly (Bonnedahl and Järhult, 2014). Reports indicate that wild birds are frequent carriers of ESBL producing *E. coli* (Alcalá *et al.*, 2016; Sandegren *et al.*, 2018). Farmed red deer and small mammals (Alonso *et al.*, 2016), and reptiles in Galapagos islands (Wheeler *et al.*, 2012), Brazilian grey-breasted parakeets (Machado *et al.*, 2018), and Mallards (Sandegren *et al.*, 2018) are reported to carry antibiotic-resistant enteric bacteria.

Stressors: Microbes are routinely exposed to various environmental or host induced stressors and they have developed strategies to overcome such adversity (Begley and Hill, 2015; Horn and Bhunia, 2018). It has been observed that bacterial exposure to various food preservation treatments including thermal or nonthermal including chemical processing treatments result in sub-lethal stress conditions which have been shown to increase antibiotic resistance in microbes (McMahon *et al.*, 2007; Verraes *et al.*, 2013; Horn and Bhunia, 2018). This is possibly achieved through the upregulation of global stress-response regulatory genes that are also involved in antimicrobial resistance (Poole, 2012; Singh *et al.*, 2015). Stressors can also induce the expression of a multidrug efflux pump (Poole, 2012), and alter antimicrobial targets (Gunn, 2001) to overcome the adverse effects of antimicrobials (Tkachenko *et al.*, 2012).

Strategies to mitigate antimicrobial resistance

The best practice would be to reduce indiscriminate use or misuse of antibiotics in animal health, human medicine and in agriculture (Wegener, 2003; Toutain *et al.*, 2016). As mentioned above, banning of certain antibiotics in animal production can also lessen the abundance of antibiotic resistance. However, the question remains, if such a strategy alone would be sufficient to reduce the global antibiotic resistance problem. Credible alternative solutions are needed since antimicrobials are necessary for food animal production. Plant or animal-derived antimicrobials, essential oils, enzymes, organic acids, prebiotics, and immunostimulants have been proposed as a substitute for antibiotics as growth promoters (Hashemi and Davoodi, 2011; Huyghebaert *et al.*, 2011; Bhat *et al.*, 2015). Development of new-generation drugs such as glycopeptide antibiotics that specifically target the cell wall biosynthesis is proposed (Sarkar *et al.*, 2017). New-generation eco-friendly antimicrobials have been proposed that would have a limited effect on the commensals (gut microbial community) or the environment (Toutain *et al.*, 2016). In recent years, however, the use of microbes (bacteriophages, microflora, probiotics) as an eco-friendly tool to defend against antibiotic resistance (Kutateladze and Adamia, 2010; Jordan *et al.*, 2014; Pamer, 2016; Wong and Santiago, 2017; Relman and Lipsitch, 2018) is gathering momentum for treatment of various diseases, as prophylactics or as growth promoters which are discussed below.

Bacteriophages: Bacteriophages are viruses that infect bacteria. They can multiply and cause lysis of the host bacterium (lytic phase)

or can be maintained as prophage (lysogen). Their action is generally species-specific without affecting non-target bacteria including commensals. The first therapeutic application of bacteriophage in humans was reported in the early 20th century (1921) by Felix d'Herelle to treat bacterial infection (cholera and bacillary dysentery) (Dublanche and Bourne, 2007). In recent years, the resurgence of multidrug resistance in pathogens has found bacteriophage as a potential therapeutic agent to replace or supplement antibiotics (Merril *et al.*, 1996; Gorski *et al.*, 2009; Kutateladze and Adamia, 2010; Kortright *et al.*, 2019). In 2006, US-FDA approved bacteriophage as a biocontrol agent to prevent *Listeria monocytogenes* in cheese and since then multiple bacteriophage preparations have received GRAS (generally recognized as safe) status for controlling *E. coli* O157, *Salmonella enterica*, and *Shigella* spp. for food safety applications (Moye *et al.*, 2018). To broaden bacteriophage activity against different species or strains of pathogens, bacteriophage cocktails are now used in food safety applications. There are several commercial phage products such as Salmonellex™, SalmoFresh™, EcoShield®, PhageGuard E™, Listex P-100™, ListShield®, and ShigaShield® are available for control of zoonotic foodborne pathogens (Goodridge *et al.*, 2018).

Bacteriophages have also found wide-spread application in livestock to reduce pathogen load in food animals (Cooper, 2016). The application of bacteriophages to control *Salmonella* and *Campylobacter* in poultry has been thoroughly reviewed (Grant *et al.*, 2016). In the majority of application trials, bacteriophages were applied through the feed to chicks as well as slaughter age chickens

that were artificially inoculated with pathogens. There was a significant reduction of these pathogens in finished birds (Carvalho *et al.*, 2010; Bardina *et al.*, 2012). The use of phage tail spike protein has been also shown to reduce *Salmonella* colonization in poultry (Waseh *et al.*, 2010).

Bacteriophages have been also used to reduce *Salmonella* colonization in swine when applied through feed (Wall *et al.*, 2010; Callaway *et al.*, 2011; Zhang *et al.*, 2015). Furthermore, direct feeding of a microencapsulated phage cocktail can also significantly reduce *Salmonella* shedding in pigs before slaughter (Saez *et al.*, 2011). In a comprehensive six-week growth trial in pigs, researchers show that bacteriophages were able to suppress *Salmonella* counts, and promoted growth similar to growth-promoting antibiotics (Yan *et al.*, 2012). Bacteriophages were also used in cattle to prevent *E. coli* O157:H7 colonization and shedding and were able to reduce bacterial load but failed to eliminate it completely (Sheng *et al.*, 2006; Wang *et al.*, 2017).

Endolysin, an enzyme produced by bacteriophage is responsible for bacterial cell lysis by hydrolyzing cell wall peptidoglycan therefore, purified endolysin has been considered a substitute for antibiotics (Borysowski *et al.*, 2006). Recombinant endolysin also show similar potential therapeutic and prophylactic effects (Fenton *et al.*, 2010).

Microbial community: It has been well accepted by the scientific community that exposure to the desirable microbial community early in life improves gut health, immune system development and infection

resistance (Fuller, 1989; Marchesi *et al.*, 2016; Marcolla *et al.*, 2019). Promoting healthy microbial community in livestock through diet, probiotics, prebiotics, and husbandry practices can reduce pathogen load (Chambers and Gong, 2011; Stanley *et al.*, 2014; Brugman *et al.*, 2018). For example, increasing propionate producing commensal bacteria in the gut has been shown to exert colonization resistance against *Salmonella* infection (Jacobson *et al.*, 2018). Animals through their natural feeding practices (such as coprophagy habit of birds) often receive fecal microbiota from their environment thus can help repopulate their diverse microbial community. However, such behavior can also allow pathogen ingestion from the environment as well.

Therefore, fecal microbiota transplantation from healthy (presumably free of zoonotic pathogens) in animals has been proposed for the control of certain diseases (Niederwerder, 2018). Direct-Fed Microbials (DFM) or Competitive Exclusion cultures (CE) have been used in poultry to reduce pathogen colonization and increased performance including meat and egg production. DFM may contain undefined cultures therefore, its use has been reduced significantly while CE containing known defined cultures are receiving greater acceptance (Callaway *et al.*, 2017). In poultry production, hatchlings (day-old chick) fed with gut microflora have shown to reduce *Campylobacter* and *Salmonella* colonization thus reducing the use of antibiotics to control zoonotic pathogens (Tellez *et al.*, 2012).

Fecal microbial implant from a healthy donor has proved to be an important therapeutic strategy for the treatment of diseases where

antibiotic therapy is ineffective (Pamer, 2016). Such an approach would also reduce the use of antibiotics thus prevent the onset of acquired antibiotic resistance. Fecal microbiota has been shown to reduce antibiotic resistance genes in human patients suffering from recurrent *Clostridium difficile* infection (Millan *et al.*, 2016). Furthermore, fecal microbiota transplants can also reduce intestinal colonization with antibiotic-resistant microbes (Crum-Cianflone *et al.*, 2015; Woodworth *et al.*, 2019). The major drawback of DFM is that the cultures are undefined, therefore, the microbial community may vary from batch to batch and may carry pathogens.

Probiotic bacteria: Probiotic bacteria consist of well-characterized defined cultures which exert health beneficial effects on the host (Amalaradjou and Bhunia, 2012; Hill *et al.*, 2014; Plaza-Diaz *et al.*, 2019). Among the probiotic bacteria, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Bacillus* and yeast (*Saccharomyces boulardi*) are widely used. They survive, colonize, and modulate the immune system in the gut, and are considered safe (Lebeer *et al.*, 2008; Amalaradjou and Bhunia, 2012; Ryan and Bhunia, 2017). Because of their competitive exclusion capacity and immunomodulatory function, these are considered an ideal substitute for antimicrobials in livestock diet (Cameron and McAllister, 2019). Therefore, the use of probiotics is one of the options being proposed by the scientific community as an alternative to antibiotics (Ouwehand *et al.*, 2016; Hossain *et al.*, 2017; Nair *et al.*, 2018).

While it is relatively easier for a bacterial pathogen to develop resistance against

antibiotics it is difficult to evolve against probiotics. This is largely due to the multiple inhibitory mechanisms employed by probiotics including the production of antimicrobial compounds (acids, bacteriocins), competitive exclusion and colonization resistance, immunomodulation and improved epithelial barrier function to prevent pathogen invasion (Pagnini *et al.*, 2010; Amalaradjou and Bhunia, 2012; Bron *et al.*, 2017; Ryan and Bhunia, 2017). Therefore, oral administration of certain competent probiotics may be a rationale and an effective alternative for preventing pathogen colonization in food animals, ameliorating disease and reducing antibiotic use (Gaggia *et al.*, 2010).

In livestock production, probiotics are commonly used for the competitive exclusion of pathogens such as *Salmonella*, *Campylobacter*, *E. coli* and *Clostridium* (Schneitz *et al.*, 2016; Hofacre *et al.*, 2019). Probiotics were also used in broilers to exclude ESBL *E. coli* (Ceccarelli *et al.*, 2017), *Salmonella* (Tellez *et al.*, 2012) and *Campylobacter* (Santini *et al.*, 2010). Besides, probiotic application also improves feed intake, and weight performance in pigs (Yang *et al.*, 2015; Pan *et al.*, 2017; Wang *et al.*, 2018), in beef and dairy cattle (Penha *et al.*, 2011; Maldonado *et al.*, 2018), in poultry (Rai *et al.*, 2013; Callaway *et al.*, 2017), in fish and shellfish (Kapareiko *et al.*, 2011; Lara-Flores, 2011; Ramos *et al.*, 2013).

Among the probiotic bacteria, spore-forming *Bacillus* species have found widespread application in livestock production and in aquaculture as a growth promoter and is considered an ideal substitute for antibiotics (Guo *et al.*, 2009; Nimrat *et al.*, 2012;

Mingmongkolchai and Panbangred, 2018). *Bacillus* spp. has been used as DFM to promote growth, immunity and as an antibiotic substitute in poultry (Grant *et al.*, 2018). The common probiotic *Bacillus* spp. include *B. cereus*, *B. clausii*, *B. pumilus*, *B. subtilis*, *B. coagulans* and *B. licheniformis* (Cutting, 2011). Their ability to form spores allows them to survive well in feed/food and the gastric passage. The spores after passing through the stomach (acid acts as a stimulant) germinate and the vegetative cells can exert their probiotic action.

Bioengineered next-generation probiotics

One of the limitations of probiotics is that the antimicrobial action may be species or strain specific. The probiotic action is largely dependent on their ability to stably colonize the gut. Adhesion to the mucosa or stable colonization and subsequent anti-inflammatory and immunomodulatory actions are crucial for probiotic health benefits (Westermann *et al.*, 2016; Yadav *et al.*, 2017; Suez *et al.*, 2019). Since not all probiotic possess such property, often, bioengineering approach has been employed to create next-generation organisms expressing desirable traits including adhesion molecules, cytokines (IL-10, IL-27), and enzymes (Amalaradjou and Bhunia, 2013; Sleator, 2015; Kumar *et al.*, 2016; O'Toole *et al.*, 2017). Such strains have shown increased ability to prevent pathogen colonization, exert immunomodulatory action and control infection. *Lactobacillus casei* strains expressing *Listeria* adhesion protein (LAP) was able to prevent *L. monocytogenes* interaction with the human intestinal epithelial cells *in vitro* and reduce *Listeria* induced cell damage (Koo *et al.*, 2012; Drolia *et al.*, 2018). Similarly, *L. casei* expressing invasion

protein, InlAB was able to reduce *L. monocytogenes* colonization (Mathipa *et al.*, 2019a; Mathipa *et al.*, 2019b). Application of such bioengineered strains in food animals should be able to increase stable colonization of probiotic in the host, improve gut barrier function and reduce the spread of zoonotic pathogens and antibiotic resistance.

Benefits of microbes as antimicrobials

- Mechanism of antimicrobial action is multifaceted thus the development of acquired resistance is very low.
- Microbes may compete for the same niche as the pathogen in the host thus can exert colonization resistance.
- Bacteriophages are natural antimicrobials and can attack target pathogens effectively and has no known side effects on the host physiology.
- Some probiotic bacteria also produce organic acids (such as lactic acid, propionic acid, butyric acid, and acetic acid), bacteriocins, and hydrogen peroxide which are inhibitory towards pathogens.
- Probiotics have an immunomodulatory action and can improve gut barrier function, therefore, can prevent undesirable bacterial translocation across the gut epithelial barrier.
- Application of spore-forming probiotics (example, *Bacillus* spp.) would ensure increased survival in feed and the gastrointestinal tract.
- Bioengineering of a strain can enhance its colonization ability in

the host or to help secrete anti-inflammatory or immunomodulatory cytokines.

Limitations of microbes as antimicrobials

- Reduced survival of microbes in the feed. The delivery strategy and extended survival of microbes in the feed should be ensured. Alternatively, spore-formers can increase survival in feed during storage.
- Some *Bacillus* spp. are known to produce toxins (enterotoxins) and antibiotics (ex. bacitracin, subtilin, etc), therefore, strains should be thoroughly screened before use.
- Mixed undefined culture (fecal implant or microflora) may carry pathogens or microbes carrying antibiotic resistance genes which may become an unintentional source of AMR.
- Antimicrobial action of bacteriophage depends on the presence of the target pathogen. In the absence of the target host, bacteriophage may disappear from the host.
- Bacteriophages may carry antibiotic marker genes or other virulence genes thus they should be thoroughly screened before application.
- The development of resistance to bacteriophages could be a problem.
- Bacteriophage production requires the growth of large volumes of host pathogens which can be hazardous

during mass production and the chance of contamination of bacteriophage preparation with the host bacterial pathogens.

- Probiotic LAB should be thoroughly characterized before application but the major limitation is its ability to stably colonize the host gut. Probiotic from a heterologous source may not colonize a host thus they may be excluded from the body without colonization and may have limited antimicrobial activity.
- Bioengineered probiotic strains can have increased colonization depending on the type of genes being introduced, however, the strain should be made in such a way that they do not survive outside the host so environmental spread can be minimized.

Indiscriminate use or misuse of antibiotics coupled with a lack of investment in developing new drugs culminated in the global antibiotic resistance crisis today. It is nearly impossible to entirely eliminate antibiotic resistance in microbes, but the clinical or animal husbandry practices that can reduce further development of acquired resistance to antibiotics should be explored. One caveat, any credible solution to reduce antibiotic resistance in bacteria should be able to withstand the resistant mechanisms used by bacteria in the first place.

Antibiotics resistome are widespread and are routinely isolated from food animals, wildlife, foods, soil, water and the environment. Modern intensified animal

agriculture practice has been identified as the major contributing factor and the banning of certain antibiotics has shown a positive result and reduced antibiotic resistome in foods and in humans. However, antimicrobials use in food animal production, poultry industry and in aquaculture are essential to treat and prevent the spread of diseases. Furthermore, agriculture scientists are continuously being reminded of growing human populations on the planet and the demand for increased food production in the next few decades. Therefore, any changes in our husbandry practices that can potentially affect food (milk, egg, meat) yield and the profit margins for the farmers would face resistance.

Alternative but equally effective as antibiotic must be developed that can replace antibiotic use in food animal production and environmental burden without affecting the animal health or growth performance. Therefore, in recent years, eco-friendly new generation antimicrobials including microbes such as bacteriophages, mixed microflora, probiotic bacteria, bioengineered microbes, microbial by-products, and plant or animal-derived antimicrobials are investigated as a potential antibiotic replacement or as an antibiotic supplement.

Besides promoting antibiotic stewardship, the total banning of antibiotics especially the “medically relevant” antibiotics for growth promotion should be enforced. At the same time, each country should make a commitment to invest funds towards research and development to produce eco-friendly antimicrobials including vaccines that are well suited for their husbandry practices for the animal, poultry, and fish production.

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