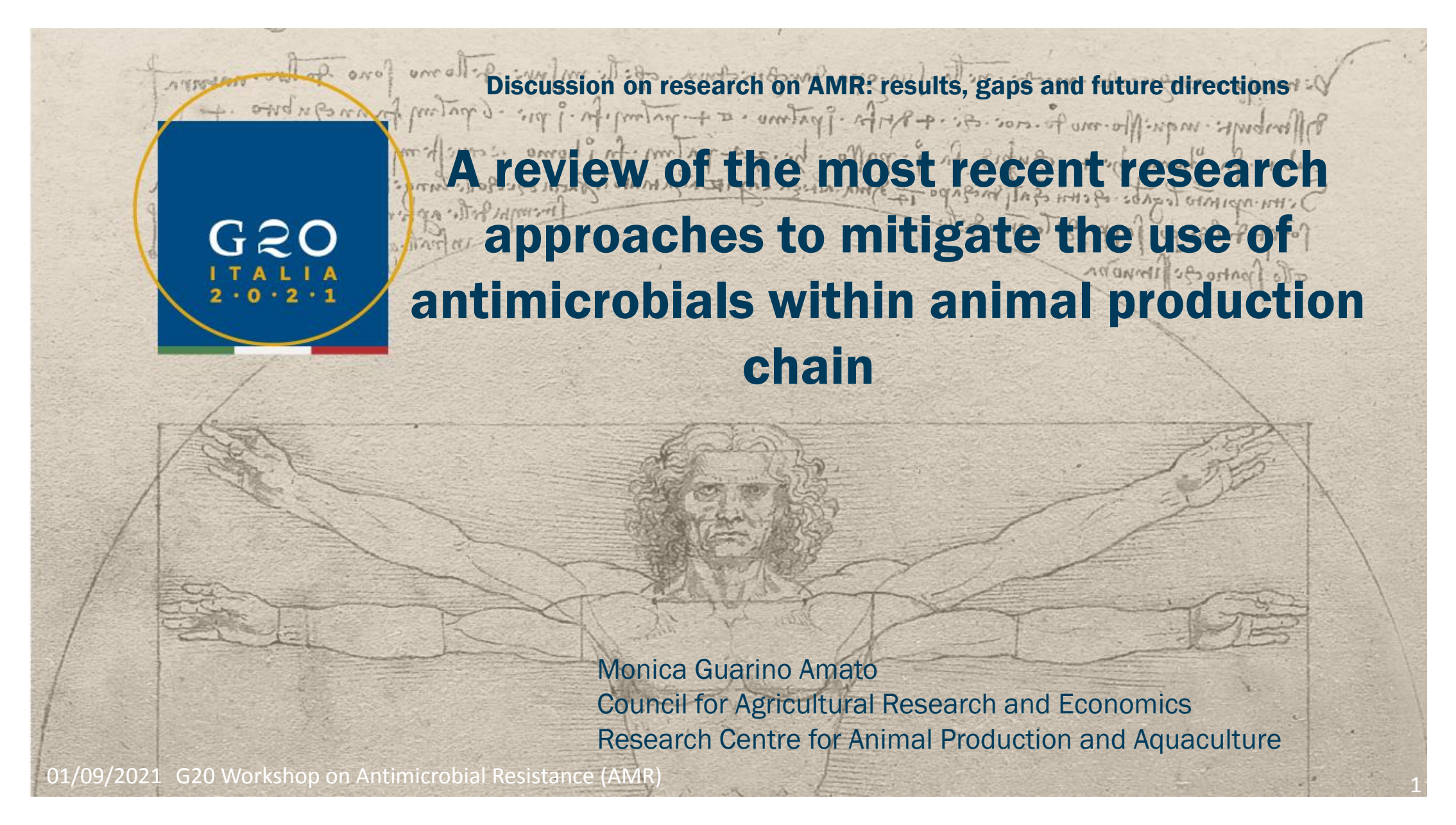
Discussion on research on AMR: results, gaps and future directions

A review of the most recent research approaches to mitigate the use of antimicrobials within animal production chain



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The threat of the emergence of a superbug is now becoming very real, and all recent updates on this issue show the increasing complexity and scope of bacterial resistance patterns. To preserve human good and the effectiveness of antimicrobial, we have to learn how to rapidly change strategies and procedures, as bacteria do, to preserve our health and wellbeing. Strategies should combine a series of different multidisciplinary approaches towards animal health and welfare.

The follow review takes into account only the last and prominent researches to find alternatives to antimicrobials.
Biosecurity and herd/flock management should be used concomitantly to reach the aim of antimicrobial reduction.

Mechanisms for bacteria virulence disruption

Targeting bacteria quorum-sensing mechanism

Targets for bacterial virulence disruption

- bacterial two-component systems,
- bacterial biofilm formation mechanisms,
- bacterial capsulation systems,
- bacterial toxins secretion systems,
- protein secretion mechanisms,
- cyclic di-GMP signaling mechanisms,
- quorum-sensing mechanisms**,** microbes use that to orchestrate collective population behavior. It depends on production and release of specific chemicals/signals. Naturally-available or synthetic compounds can be utilized to control bacterial pathogenesis. Chemicals used in quorum-sensing disruption could have some off-target capabilities and could possibly attenuate the activities of some desirable bacterial species, e.g. lactic acid bacteria

Chemicals

Organic acids and their derivatives

- Antibiotic growth promoters (AGPs) have been used in the animal industry for several decades in various countries, to improve the production rate and reduce mortality In recent years, organic acids gained attention as a possible alternative for AGPs, especially in the poultry industry.
- Organic acids demonstrated great potential as antimicrobial agents
- Organic acids used in poultry were reported to exhibit antimicrobial actions against a wide range of intestinal pathogens, enhance nutrient metabolism, and improve performance.
- The efficacy of an organic acid is influenced by several factors such as the chemical composition and form, pKa-value, molecular weight and targeted microorganism control.
- Further studies with available molecular tools are needed to specifically understand the genetic mechanisms that regulate bacterial response towards organic acids before developing any commercial/empirical applications at the farm gates.

Chemicals

Metals and minerals

- Copper, zinc and silver have antibacterial activity, it can disrupt bacterial protein functions, generate reactive oxygen species and causes damage to bacterial DNA. Copper and Zinc salts are commonly added to animal feeds in concentrations above dietary requirements to improve growth. Metal nanoparticles such as silver, copper oxide, and zinc oxide are of particular interest for their antimicrobial properties.
- Zinc oxide has been successfully used in animal feed at a large-scale to boost growth. Zinc oxide is currently used extensively in Europe as an alternative for colistin in weaned piglets and as a feed ingredient to improve feed conversion and consequently growth.
- Criticisms about propensity of the used heavy metals to to accumulate in the soil resulting in serious environmental and health consequences.
- Risk of co-resistance selection through the use of heavy metals as most of the metal resistance genes co-localize to the same genetic elements as certain antimicrobial resistance genes.

Phytochemicals

Natural compounds/secondary metabolites from plants

- Certain phytogenic compounds (and their blends) have been reported to have promising anti-microbial properties because of their abilities to increase the permeability of bacterial cell membranes and/or kill certain bacterial species.
- Few selected phytochemicals attracted the attention of the animal industry as potential alternatives to antibiotics/growth-promoters. Many studies highlighted the beneficial effects of phytochemical supplementation on animal productivity, especially in broiler chickens.
- In addition to their antimicrobial capabilities, some phytochemicals exert anti-oxidative and anti-inflammation functionalities
- Polyphenols are one of the most important groups of natural antioxidants found in plants, some of them have antimicrobial properties

Synthetic polymers and nanoparticles

Antimicrobial nanoparticles and antimicrobial-polymers

- Developing synthetic antibiotic alternatives which bacteria are less likely to develop resistance and fabricating new nano-carriers to boost the therapeutic efficacy of existing antimicrobial molecule with an increased selectivity
- Antimicrobial polymers are very promising alternatives to antibiotics for veterinary medicine
- Nano-carriers can be used to not only to boost the therapeutic efficacy of existing antimicrobials but also to convert potent broad-spectrum non-selective biocides into selective ones.
- Understanding the mechanism of bacterial growth-inhibition of many novel synthetic polymers and nanoparticles should provide a safe, effective, and inexpensive way to control the prevalence of antibiotic resistance in the animal production chain.

Enzymes

Possible alternatives to antibiotic use as growth promoters

- Exogenous enzymes have been proposed as possible alternatives to antibiotic growth promoter for several decades.
- The use of enzymes (including phytase) can improve the integrity of intestinal mucin, increase gastric residency of feed, reduced inflammatory responses and other beneficial effects on immune function and resilience.
- Supplementation of poultry diets with exogenous enzymes has a range of digestive, physiological, microbiological and immunological effects.

Herbs, spices, plant extracts and/or essential oils

Natural alternative to growth promoter antibiotics

- Plant extracts and essential oils have shown antimicrobial, antioxidant, antiparasitic, anti-inflammatory, antidiarrheal and antifungal properties, improve food conversion and stimulate digestive enzymes when they are used in animal diets.

- High antioxidant activity and total phenol concentration have been suggested as an initial screening criterion to find natural sources of dietary additives from agroindustrial waste.

- Some plant materials that can be considered as substitutes for growth promotion antibiotics because of their antimicrobial activity by the presence of polyphenolic compounds and essential oils, like garlic, oregano, thyme, moringa solanum spp.

Bacteriophage

Virus killers

- Bacteriophages are viruses that can infect and kill the bacteria without any negative effect on human or animal cells used to overcome the problem of microbial resistance. Phage therapy is not widely used currently and is approved in few countries, it has the potential to control the colonization of E.coli, Salmonella spp and Campylobacter spp in chicken.
- Further studies are needed to test the efficacy of bacteriophage treatments against various pathogens under different conditions.
- The potential of this promising biological intervention technology against the emergence and dissemination of antimicrobial resistant phenotypes as well as human and zoonotic pathogens has large future applications.

Prebiotics and probiotics

Alternatives to antibiotics used for growth promotion

- Despite the proven biological properties of probiotics, such as antimicrobial activity, research in this area is still incipient and needs further discussion
- Probiotics are natural feed additives used to improve health and growth performance.
- The use of probiotic microorganisms is intended to support the health of the host. The literature shows a large number of studies using probiotics, but most of them explain only how probiotics can maintain the intestinal health of the host. The mechanisms of action of probiotics are various, such as the production of inhibitory substances, such as bacteriocins and hydrogen peroxide, which inhibit Gram negative and Gram positive pathogenic bacteria; blockage of adhesion sites; compe- tition for nutrients
- Development of genetically engineered yeast and bacterial cells expressing antibacterials may have potential as probiotics

Bacterocins

Ribosomal synthesized peptides

- Bacteriocins are a heterogeneous group of ribosomally synthesised antimicrobial peptides with the ability to kill closely related (narrow spectrum), or a diverse range of (broad spectrum), microorganisms. Very potent, being active at nanomolar concentrations, and exert their killing effect predominantly through membrane permeabilization
- They are produced by certain bacteria
- They have bactericidal effect (e.g., causing cell death) or a bacteriostatic effect (e.g., inhibiting cell growth).
- Most of the attention has been directed towards the lactic acid bacteria as they are prolific producers of antimicrobial peptides.

Peptide production

Recombinant DNA technology

- Recombinant DNA technology enables the scalable, sustainable and cheaper production of peptides when compared to other options. Genes with different characteristics and origins are cloned into certain vectors for expression in prokaryotic or eukaryotic hosts. In recent decades, several expression systems such as bacteria, yeast, mammalian cells, insect cell cultures and plants have been used for heterologous production of molecules with pharmacological and industrial interest.

Biocins

Non Ribosomal peptides

- NRPs are known to present activity against Gram-positive and Gram-negative bacteria including multidrug-resistant strains and fungi.
- Could be produced in large scale in a specific isoform utilizing a host organism similarly to ribosomal peptides.
- Directed mutations in order to reduce cytotoxicity or increase antimicrobial activity to obtain new molecules with specific changes in their structure through.
- Prospects of NRPSs produced directly from environmental DNA or improving well-known compounds via heterologous expression are still challenging as the yield achieved in a heterologous host is often lower than in the native producer

Immune modulation approaches

Immune modulation of the host and immune derived therapeutics

- Pathogen-specific antibodies in animal feeds to activate antigen presenting cells stimulating immune responses to enhance resistance and treat disease
- Identification of NK-lysin from a chicken intestinal cDNA library led to synthetic peptides that had direct killing activity on parasites like Campylobacter sp.
- Interleukins (ILs) and interferons (IFNs) are cytokines produced by a variety of cell types that stimulate development and differentiation of cells of the immune system or induce protective responses to pathogens such as bacteria and viruses

Gut microbiome and immune development

Improved health and welfare

- Resident microbes of the gastrointestinal system have become the subject of extensive investigations and it is becoming increasingly recognized that gastrointestinal organisms play important roles in health and disease.
- Poultry have become one of the most prominent sources of animal protein worldwide, so chicken gastrointestinal microbiome is of major interest to investigators attempting to improve growth, health and food safety of poultry. There appears to be a decrease in microbial diversity of the chicken gut at 14–16 days post-hatch that is associated with an alteration from skeletal to muscle growth. Also, growth performance may differ between chicken breeds that could be associated with the gastrointestinal microbiome.

Vaccines

Vaccines as alternatives to antibiotics for food producing animals

- New technologies and approaches such as reverse vaccinology, novel adjuvants, structural vaccinology, bioconjugates and rationally designed bacterial outer membrane vesicles together with progress in polysaccharide conjugation and antigen design, are promising for the future of vaccine research and development.

- Key research breakthroughs and a number of highly promising vaccination approaches are already in development. These include new oral vaccines based on bacterial spores, live vectors, or new delivery strategies for inactivated oral vaccines; they also include new vaccination strategies in-ovo, combination vaccines that protect against multiple pathogens, the use of recent biotechnological advances, and comprehensive approaches to manage diseases caused by ubiquitous pathogens.

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