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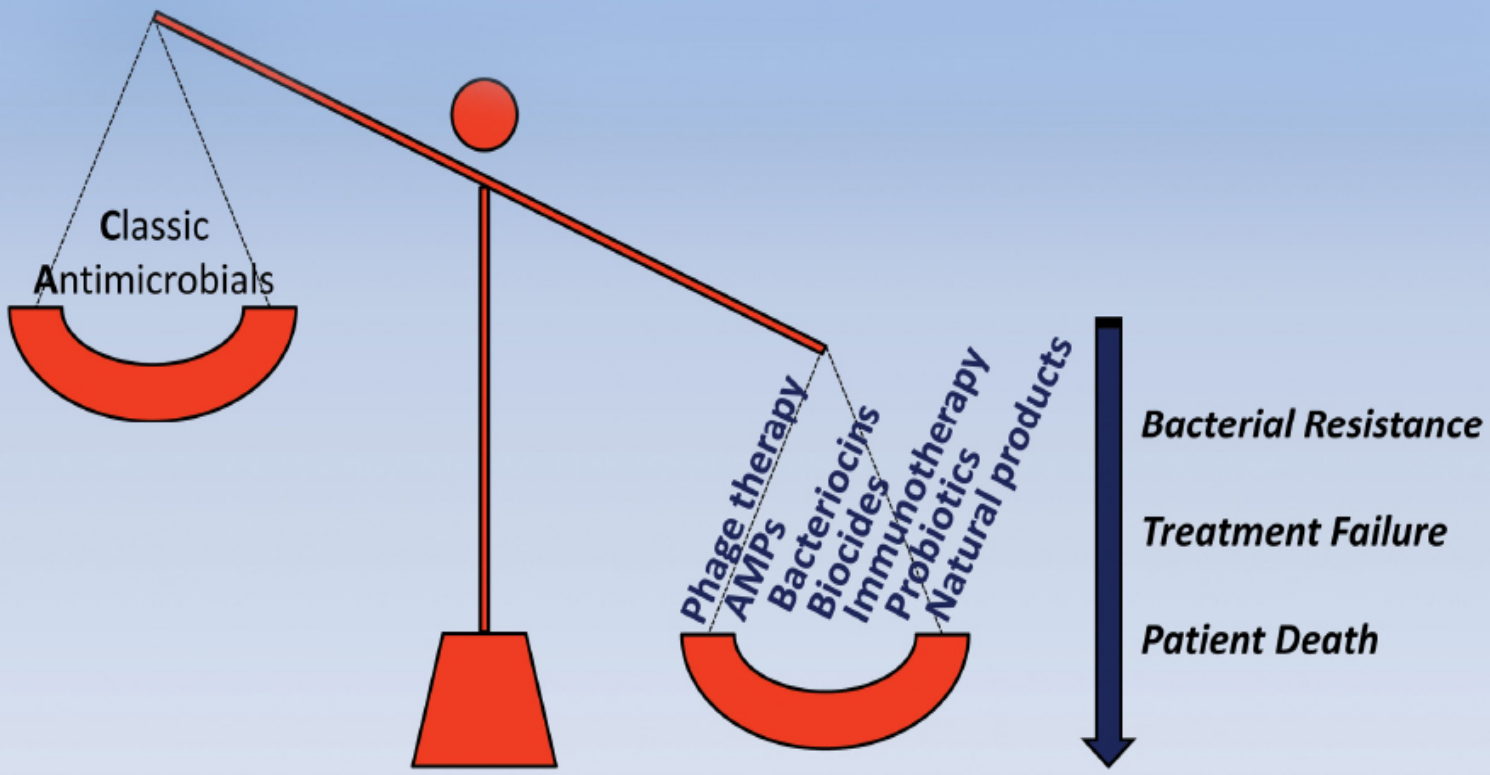
FRONTIERS IN ANTIMICROBIAL AGENTS

THE CHALLENGES OF ANTIBIOTIC RESISTANCE IN THE DEVELOPMENT OF NEW THERAPEUTICS

VOLUME 1



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Frontiers in Antimicrobial Agents
(Volume 1)

The Challenging of Antibiotic Resistance in the Development of New Therapeutics

Editors

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FOREWORD

The rapid development of highly effective antimicrobial agents during the 20th century revolutionized the treatment of diseases caused by bacteria, viruses and fungi; leading to the notion that "it was time to close the book on infectious diseases". However, today we are facing pandrug-resistant microorganisms, and antimicrobial resistance constitutes one of the major public health problems worldwide. Allied to this, the new antimicrobial agent's development pipeline is at its all-time low; because of scientific, economic and regulatory hurdles.

While we must conserve the antimicrobials we have left by using them optimally, the process of developing new agents must also be accelerated. This will hopefully facilitate targeted therapy, improving therapeutic efficacy and decreasing antimicrobial resistance.

This book describes cutting-edge research on innovative alternatives to classical antimicrobial therapy – bacteriophages, antimicrobial peptides, probiotics, immunomodulators, natural compounds, bacteriocins and biocides – and the most appropriate approaches to control the spread of drug-resistant microorganisms.

Interestingly, this book is edited and partly written by scholars dedicated to Microbiology from the area of Veterinary Medicine. This is not surprising, because the amount of antimicrobials marketed for use in animals is approximately four times greater than the quantity used in human medicine. Furthermore, the widespread use of antimicrobial agents in animal production – often administered in lower doses and for longer periods of time – has been linked to the development of antimicrobial resistance.

While the novel therapeutic strategies in Veterinary Medicine have been a major focus in the last chapter, the book has input from a wide range of experts in different disciplines – from basic science to human clinical microbiology – and truly reflects the 'One-Health' approach which spans humans, animals and the wider environment.

One final note to remember by the enthusiastic reader is that, bacteria have shown, in this continuous "arms race", that they can develop resistance to virtually all therapeutic agents. Therefore, it is very important to continue to use both antibiotics and their alternatives rationally and judiciously.

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PREFACE

This eBook was conceived after an invitation by the Bentham Science Publishers due to the necessity to review the alternative approaches to the classic antibiotic therapies, and to disperse the shadows over the use of these innovative medicines.

The prevalence of drug-resistant microorganisms has increased worldwide. However, in the last years, few novel entities to fight drug resistant microorganisms have entered the clinic. The antibacterial pipeline is scarce because of the costs associated with the clinical trials and licensing of the antibiotics. Therefore, the quest for alternatives to the classic antimicrobial therapeutics that are effective against drug resistant microorganisms is a timely and very important issue in modern medicine.

The development of novel entities to control the dissemination of drug resistant microorganisms would decrease the morbidity and mortality caused by these disease causing agents, and consequently the economic burden associated with health treatments. Some of these drugs are considered extremely valuable in the nearly future due to their low toxicity, capacity for large scale production, and most importantly their low probability to generate resistance. However, the fear associated to the use of innovative medicines such as phage therapy must be overcome. It will take time to bring phage therapy and other approaches to practice with safety, and to change mentalities of clinicians and the general public. The implementation of such innovative medicines will lead to a decrease in the antimicrobial resistance and related failure treatments. This progress will require contribution of different levels of interdisciplinary knowledge: from researchers to public health entities, from producers to consumers, including politicians.

This eBook aims to contribute to an integrated understanding concerning innovative alternatives to the classical antimicrobial therapeutics. It is based on cutting edge research and the outcome will shed light on the most appropriate approach to control the dissemination of drug resistant microorganisms. It gathers a wide range of topics on the subject and includes several chapters with original material. Authors and co-authors represent a multidisciplinary team that includes scientists and professors with a vast experience in the area, from different universities and research institutions. It is an attempt to encourage the implementation of alternative approaches to the classic antimicrobial therapeutics in human and veterinary health programmes.

The eBook is organized in nine chapters: the general introduction is followed by a review devoted to Phage Therapy, Antimicrobial Peptides (AMPs), Probiotics, Immunotherapy, Natural Compounds, Bacteriocins, Biocides, and lastly to the Novel Therapeutic Strategies in

Veterinary Medicine.

Finally, we would like to thank all authors that have enthusiastically contributed to this eBook, and all people that somehow helped us to bring it to daylight, including our family, friends, colleagues and students.

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CHAPTER 1

Introduction

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Abstract: Antibiotherapy protocols for infectious disease control were first applied in 1940s with penicillin. Although they led to a major decrease in morbidity and mortality rates, they were also responsible for the rapid emergence and dissemination of multi-drug-resistant bacteria (MDR). This phenomena led to the prompt development of new antimicrobial compounds, which soon became ineffective due to bacteria ability to develop resistant traits through mutations or resistance genes transfer.

However, the development of new approaches for prevention and control of emerging infections remains one of the major priorities and challenges for Research and Innovation (R&I). The worldwide mortality rate due to infectious diseases keeps exponentially increasing, not only due to MDR bacteria dissemination, but also due to the decline in the development and commercialization of new generations of antibacterial compounds.

Considering the "One Health" concept, new antibacterial strategies are urgent, for human and veterinary medicine. Several R&I approaches are being followed, including phage therapy, antimicrobial peptides and bacteriocins, probiotics, natural compounds, immunomodulation *via* vaccination and biocides.

Keywords: Antimicrobial peptides, Antimicrobial resistance, Bacteriocins, Bacteriophages, Biocides, Biofilm, Generally Recognized as Safe, Host, Immunomodulation, Lytic, Multi-Drug-Resistant, Natural compounds, Nisin, "One Health", Pathogens, Probiotics, Research and innovation, Therapeutic strategies, Vaccination, Veterinary Medicine.

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INTRODUCTION

Antimicrobial resistant bacteria dissemination poses an emergent challenge for the development of new antimicrobial compounds [1], which has significantly diminished in the last decade. Therapeutic options to control the dissemination of resistant bacteria are progressively decreasing. Considering the "One Health" concept, new antibacterial strategies are urgent for human and veterinary medicine. Novel therapeutics strategies aiming at infectious diseases control include phage therapy, probiotics, immunomodulation *via* vaccination, natural compounds and biocides and bacteriocins and antimicrobial peptides.

Antimicrobial peptides (AMP) may constitute new potential antimicrobial compounds: they are able of eliminating a broad spectrum of microorganisms; resistance to AMP was rarely observed; and they have the ability to modulate innate immune response [2]. AMPs are a diverse group of molecules with cationic and amphipathic properties which selectively target membranes rich in negatively charged phospholipids and no cholesterol, such as those of microorganisms [2, 3]. Most have amino acids with two types of side chains: cationic, such as arginine (R), lysine (K), and histidine (H); and bulky nonpolar, *e.g.*, proline (P), phenylalanine (F), and tryptophan (W). The former presumably mediates interactions with bacterial membranes and/or cell walls, including lipo-polysaccharide [4], whereas nonpolar chain mediates lipophilic attachment leading to membrane disruption [2, 3].

Recently, it was established that short AMPs having sequence (RW) n-NH2, (n= 2 to 4) are the optimal choice against bacteria. Hexameric and octameric peptides are potent biofilm inhibitors and octameric peptides also disperse existing biofilms and kill detached cells [3, 4]. Other advantage of short AMPs is their minimum damage to host cells, being candidates to large-scale production [3]. In addition, it was shown that some substances that disturb biofilm matrix make them more susceptible to subsequent treatment with antimicrobials or disinfectants, namely furanone [5], nisin [6], and salicylic acid [6]. Cellulase also inhibits biofilm formation by Gram-negative bacteria, probably by degrading the exopolyssacharide [7].

Bacteriocins are an heterogeneous group of ribosomally synthesized AMP produced by bacteria, divided in several classes [8]. Nisin is a class I bacteriocin produced by *Lactococcus lactis* that has antibacterial activity against a wide range of Gram-positive bacteria [9 - 11]. Nisin is already approved as a food preservative in EU (as additive E234) and USA [9].

Some bacteriocins are produced by probiotic strains. Probiotics are defined as "live microorganisms whose when administered in adequate amounts confer a health benefit on the host". Probiotics are documented to reduce or prevent specific infectious diseases of the gastrointestinal tract. Proposed mechanisms by which probiotics can inhibit pathogens in the gut environment include (i) competitive exclusion by competition for binding sites or stimulation of epithelial barrier function; (ii) stimulation of immune responses *via* increases of sIgA and anti-inflammatory cytokines and regulation of proinflammatory cytokines; (iii) direct antimicrobial activity through production of antimicrobial substances, including bacteriocins and other antimicrobial peptides (AMPs); (iv) production of biosurfactant molecules with antiadhesive and sometimes also antimicrobial activity; (v) inhibition of key cellular processes in gastrointestinal pathogens such as virulence gene or protein expression, quorum sensing or biofilm formation [12, 13].

Bacteriophages are ubiquitous bacterial viruses [14 - 20] that specifically invade several bacterial genera [21, 22]. They are obligate parasites which nucleic acid codes for the proteins necessary for its replication within the host bacterium [14] and can be lytic or temperate [14, 16, 20 - 22]. Bacteriophages used in therapy are usually lytic. Their life cycle includes adsorption to a specific phage receptor on the bacterial cell surface, genomic material injection, exponential phage replication using the host cell machinery, bacteria lysis and release of progeny phages [14, 16, 18, 21, 22]. These phages can present two bacteriolytic mechanisms, promoting cell wall hydrolysis *via* a virolysin-holin system present in most lytic phages, or *via* a single lytic factor [20].

One main advantage of phages is their host specificity [15 - 18, 20, 22], which renders them ideal for eliminating only pathogenic bacteria, belonging to a specific species, serovar or serogroup [14, 16, 20, 22]. Unrelated commensal

CHAPTER 2

Bacteriophages as Antibacterial Agents: Why are We Facing an Antibiotic Crisis and How Could Bacteriophages be of Help?

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Abstract: Bacteriophages are viral, natural and bacterio-specific entities. As a major part of the biosphere, they were involved in the origin of life. They still play an essential role in evolution and are highly involved in the development of molecular biology. In the issued biotechnology industry, they are promising as a sustainable antibacterial. Felix d'Hérelle, one of the discoverers of bacteriophages, first proposed "(bacterio) phage therapy" in the early 20th century. At the Eliava Institute in Tbilisi, Georgia it was further developed and it is still used in medical practice in all the former Soviet Republics. The Western world, with the advent of antibiotics, almost forgot phage therapy.

The antibiotic resistance crisis placed phage therapy again in the spotlights. The main problem today is the lack of evidence based therapeutic phage studies in accordance to modern standards as well as the lack of an adapted phage therapy regulatory frame. Initiating clinical studies in this context is difficult. Phage therapy is sporadically applied today, although under specific conditions like the Helsinki Declaration and/or specific national regulatory frames (Poland). This impedes clinical application and scientific progress.

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However, several groups setup animal and human studies, while the idea of using bacteriophages as antibacterial is already applied in the food industry. In the clinic application seems imminent. Several issues, also from the fundamental scientific perspective, still need to be tackled while practically an adapted regulatory frame is urgently needed.

Keywords: Antibiotic crisis, Antibiotic resistance, Bacterial host, Bacteriophage, Diarrhoeal diseases, Eliava Institute, *Escherichia coli*, Food and Drug Administration, Holins, hormesis, *Listeria monocytogenes*, Phage cocktail, Pharmaceutical industry, *Pseudomonas aeruginosa*, Public health, Regulatory obstacles, Safety, Seed lots, *Staphylococcus aureus*, Therapy.

1. INTRODUCTION

Antibiotic resistance (ABR), although not yet fully characterized neither understood, is not a newly discovered biological phenomenon. It is a fact, worldwide, that has a huge negative impact on our societies.

Already Alexandre Fleming, the discoverer of penicillin and initiator of the antibiotic era, noticed the development of ABR. He effectively described ABR and warned the scientific and medical community for this phenomenon in his Nobel Prize lecture in 1945. That was 70 years ago! [1]. ABR originated when bacteria emerged and co-evolved. The discovery and further development of antibiotics brought “western” medicine to the highest level ever. Indeed at the end of the sixties and during the seventies, society and medical officials thought that they could close the book of infectious diseases [2]. However, microbes, such as bacteria, are everywhere and interact with all other living beings. The Spanish microbiologist Baquero and his group described ‘antimicrobial resistance’ as a ‘typical emerging characteristic of a dynamic, highly complex and self-organizing system evolving at the edge of chaos’ [3]. Actually, we realize that using antibiotics, once seen as ‘miracle drugs’, in the way we did and that we are still doing, has resulted in a bacterial response we did not expect. Human interventions in different interconnected fields like human and veterinary medicine, agriculture, the food industry sector, global travel/transport of humans and animals, have accentuated and concentrated the ABR phenomenon worldwide [4]. Unfortunately, we did not do very much to try to minimize or prevent the emergence of

enhanced ABR. It is now realized that the ABR phenomenon is not yet completely described and understood. Antibiotics can naturally fulfill different concentration dependent functions (hormesis), among the bacterial communities [5 - 7]. Renowned global and national organizations are just beginning to get grips on the ABR problem [8, 9]. Among the actions and pathways that have recently been proposed to deal with ABR, *one* could be of timely help and this in a sustainable way: phage therapy, or the use of bacterio-specific viruses (bacter - iophages) as antibacterial agents.

In this chapter, we would like to shortly describe the reasons why we are facing the antibiotic crisis and how the bacteriophage approach could be of help in resolving this crisis.

2. WHY ARE WE FACING AN ANTIBIOTIC (AB) CRISIS?

Several causes can explain why we are facing this AB crisis. First, fundamentally, there is still a lack of knowledge of the role of antibiotics in nature and an insufficient integration of the notion of evolution in medicine and in our society as a whole [10, 11]. Second, our actual narrow and too mechanistic view of society and the natural environment, resulted in the over-and misuse of antibiotics in medicine, agriculture, cattle breeding and food production. Why, for example, are we regularly facing very costly epidemics in poultry, cattle or pork meat production? Because farming animals are breed in crowded artificial environments, using clonal species, which does not take into account the fact that variability and diversity is the base of sustainability.

Nature is complex and involves highly dynamic interactions between all its hierarchical structured elements on which evolution interacts.

Yet, man tried to use an antibiotic, a stable static chemical substance, against a living and evolving entity (a bacterial cell). As we know today, and as could have been expected, this resulted in the emergence of bacterial populations that are resistant against that substance. Man continued along the path and used newly discovered or natural or synthetic antibiotics to substitute those antibiotics that had become unusable.

CHAPTER 3

Antimicrobial Peptides

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Abstract: Most Antimicrobial Peptides (AMPs) are small peptides with 10–40 residues. They are part of the immune system in virtually all multicellular organisms as a defense strategy. They are promising drug candidates for the control or treatment of several diseases or to be used in combination with conventional therapy. There are several AMPs effective against cancer cells and diseases caused by bacteria, fungi and parasites. However, none AMP is currently commercialized due to a number of reasons such as high production costs as compared to conventional antibiotics. The new generation of AMPs, mainly antimicrobial peptidomimetics, has several drug candidates currently in Phase I and II clinical trials. Understanding the AMPs' fully mechanism of action is crucial to develop AMPs into useful antimicrobial agents highlighting their strengths: broad-spectrum antimicrobial activity, low probability to generate resistance, and potential for topical and injected applications.

Keywords: Amphipathicity, Anticancer, Antimicrobial peptides, Antiparasitic, Antiviral, Cathelicidin, Cationicity, Conventional antibiotics, Databases, Defensins, Dissemination, Drug development, Immune system, Interaction, Microorganisms, Multiresistance, Peptidomimetics, Permeabilization, Pharmaceutical companies, Therapy.

INTRODUCTION

The prevalence of drug-resistant microorganisms has increased worldwide [1]. Only two novel classes of antibiotics to fight drug resistant microorganisms have entered the clinic in the past three decades [2]. Moreover, the antibacterial pipeline is scarce because of the costs associated with the clinical trials and

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licensing of the antibiotics [2]. The emergence of multiresistant strains and the temporal and geographical variations in resistance patterns suggest the need to look for alternatives to the classic antimicrobial therapy to decrease the morbidity and mortality caused by these disease causing agents. AMPs may act as lead molecules for the development of novel anticancer agents and antimicrobial agents with a broad-spectrum antimicrobial activity displayed at micromolar concentrations or less, and low probability to generate resistance [3]. However, there are some limitations in the application of antimicrobial peptides that calls into question if they will be the raising stars for tomorrow's clinical practice.

1. WHAT ARE ANTIMICROBIAL PEPTIDES (AMPS)

Most Antimicrobial Peptides (AMPs) are 10–40 residue polypeptides. However, some may have more than 100 amino acids. The amino acid sequence of AMPs is part of the genetic code of virtually all multicellular organisms. They are part of the immune system across eukaryotes as a defense strategy, providing a first barrier against dissemination of a wide range of microorganisms, including bacteria, virus, protozoa, and fungi [4, 5]. The antimicrobial activity is derives from their cationic charge due to the presence of multiple lysine, tryptophan, and arginine residues, a large portion of hydrophobic residues (50% or higher), hydrophobicity, and amphipathicity [6].

A single peptide may present multiple functions according to the concept of promiscuity. They may act over different targets depending on their physical-chemical properties (pure promiscuity) or on their amino acid modification (family promiscuity) [7]. Therefore, AMPs exhibit physiological advantages over other molecules for application in the drug discovery and development process. They are promising drug candidates for the control or treatment of human, animal, and plant (agricultural) diseases, or to be used in combination with conventional therapy.

1.1. Mode of Action

It has been generally accepted that the mode of action of AMPs occurs through membrane permebilization owing to the hydrophobic nature of cellular membranes, and less commonly through non-membrane disruption (non-lytic) [6, 8].

Membrane permebilization can either consist in pore formation in the lipid membrane (barrel stave and toroidal pore models), thinning of the membrane bilayer, membrane dissolution (carpet model), or lipid-peptide domain formation [9]. Membrane is where AMPs action begins. The AMPs membrane, cationic and hydrophobic, initially interacts to the anionic lipid head groups of the outer surface of the microbial cytoplasmic membranes which are rich in lipids such as phosphatidylglycerol or cardiolipin. The difference in the exposed lipid content in the membranes of microbes and host cells is the most used explanation for the selectivity of AMPs for microbes over host cells [10]. Therefore, the binding of AMPs to the membranes of microbes is stronger than to the neutral animal plasma membranes rich in phosphatidylcholine/cholesterol/sphingomyelin [10].

After interaction, the peptide is inserted into the outer leaflet of the membrane, roughly parallel to the bilayer, leading to the displacement of lipids and perturbing the membrane. Following insertion into the bilayer, AMPs dissipate the electrochemical gradient across the microbial membrane. Experimentally, this happens within a few seconds after addition of AMPs [11, 12]. Finally, the disruption of the membrane structure occurs (membrane blebbing, vesiculation, fragmentation, DNA release, and cell aggregation) leading to the disruption of the microbial cell morphology [10]. AMPs should be able to rapidly cross through the bacterial cell walls, crossing the thick proteoglycan layer of the Gram-positive bacteria and the layer of LipoPolySaccharide (LPS) in the outer membrane of Gram-negative bacteria.

In non-membrane disruption, AMPs translocate into the cytoplasm and attack internal cellular targets affecting cellular processes from RNA and DNA synthesis [13] to loss of ATP from actively respiring cells [14].

It is likely that most AMPs show several simultaneous mechanisms of action such as membrane permeabilization and intracellular effects. This would help explain AMPs broad-spectrum activity and the scarcity of bacterial resistance to AMPs [10].

Because of the AMPs' ability to translocate into the cytoplasm, membrane-crossing AMPs have also been used as templates for the development of cell-

CHAPTER 4

Probiotics: Ways of Action and Beneficial Effects

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Abstract: Probiotics are defined by Food and Agriculture Organization (FAO) and World Health Organization (WHO) as: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". *Bifidobacterium* and *Lactobacillus* are the principal probiotic groups; however, there are reports on the probiotic potential of *Enterococcus, Bacillus* and yeasts. A number of potential benefits of probiotics have been proposed, including: alleviation of lactose intolerance, immunomodulatory activity, hypocholesterolaemic effects, prevention of inflammatory bowel disease and effect on *Helicobacter pylori* eradication. Currently, most of probiotic strains used commercially were initially isolated from human feces whether adults or children, as well as dairy products. However, recently, it has been shown that non-fermented dairy foods such as vegetable foods are an excellent source of bacteria to find new probiotic strains with a significant probiotic potential. In this review, the history of probiotics, their mechanisms of action and the beneficial health effects are presented.

Keywords: *Bacillus*, Beneficial health effects, *Bifidobacterium*, *Enterococcus*, Immunomodulation, *Lactobacillus*, Mechanisms of action, Metabolic effects, Probiotics, Safety assessment, Technological and functional criteria, *Sacharomyces*.

1. INTRODUCTION

The term probiotic comes from the Greek 'pro bios' which means 'for life'. The interest for probiotics began with the history of man; fermented milk and cheese were well-known to the Greeks and Romans, who suggested their consumption, especially for convalescents and children. Introduction of the concept is generally

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attributed to Eli Metchnikoff, who in 1907 observed that Bulgarian peasant populations lived to very old ages; they had average life spans that stretched longer than wealthier European populations [1]. Their diets, Mechnikov annotated, were rich in yogurt and other fermented milk products [2].

Eli Metchnikoff related this to the 'Bulgarian *bacillus*' which he later demonstrated how healthy bacteria in yogurt helped digestion, improved the immune system and counteract the putrefactive effects of gastrointestinal (GI) metabolism [3]. His work at the Pasteur Institute in Paris reinforced his theories about the beneficial qualities of the lactic acid bacteria (LAB) produced by fermentation. He called the strain *Lactobacillus bulgaricus*. Mechnikov and his colleagues began drinking sour milk to populate their own gut flora, in this way introducing the modern probiotic [4].

The scientific rationale for the health benefit of lactic acid bacteria was given in his book "The prolongation of life" published in 1907 [5]. He suggested that the bacteria implicated in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, remove the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health [4]. Mechnikov in his book affirmed that some of the microorganisms present in the large intestine were a source of toxicants, that contributed to disease and aging. He found that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the prejudicial microbes by useful microbes" [5].

At the same time, another important discovery was made at the Pasteur Institute by Henri Tissier who demonstrated that children with diarrhea had a low number of bacteria characterized by a peculiar, Y shaped morphology. These "bifid" bacteria were, on the contrary, abundant in healthy children [6]. He found that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora. The works of Metchnikoff [1] and Tissier [6] were the first to make scientific suggestions concerning the probiotic use of bacteria.

According to Vergin [7], the term 'Probiotika' was originally coined by the physician and dietician Werner Kollath shortly after the Second World War,

meaning ‘food ingredients with health-promoting characteristics beyond their nutritional value’ [8]. Vergin [7] suggested that the microbial imbalance in the body caused by antibiotic treatment could have been re-established by a probiotic rich diet; a suggestion cited by many as the first reference to probiotics as they are defined at the present time. Similarly, Kolb [9] recognized prejudicial effects of antibiotic treatment and proposed the prevention by probiotics [2].

Later and independently, Lilly and Stillwell, introduced the term ‘probiotic’ into the international literature in 1965, saying that a probiotic is a microbial substance secreted by one microorganism which stimulate the growth of another, thus being the exact opposite of an antibiotic [10]. Nevertheless, this definition did not gain wide acceptance and failed to survive. Since that time, the meaning of the term ‘probiotic’ has changed considerably. In 1974, Parker talks about a food supplement for livestock and improved name of probiotics as "organisms and substances which contribute to intestinal microbial balance” [11].

Some other definitions followed, with Fuller [12] being the first pointing out the microbial nature of probiotics by redefining the word “probiotic” as “a live microbial feed supplement that beneficially affects the host animal by improving its intestinal balance”. Havenaar and coworkers [13] defined probiotics as ‘mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the property of the indigenous flora’. Guarner and Schaafsma [14] gave a more recent definition as “live microorganisms, which when consumed in adequate amounts, confer a health effect on the host”.

The report of the joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) redefined probiotics as: ‘‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’’ (FAO/WHO) [15] (Table **1**). In 2002, the United Nations FAO/WHO Working Group generated new guidelines for the development and evaluation of probiotics found in foods (Fig. **1**).

CHAPTER 5

Immunotherapy

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Abstract: The relationship between a host and a pathogen is dynamic, and its outcome depends on the virulence of the invader and the relative degree of resistance or susceptibility of the host at that particular occasion. Fortunately, only an infinitesimal minority of microorganisms are able to avoid the host defenses and eventually cause disease. The host has evolved multifaceted strategies for defending itself against invasion, and pathogens have evolved their own strategies of counter-attack for host defenses pointing us to the co-evolution of "defense" and "attack" mechanisms. Many factors determine the outcome of the bacterium-host relationship. In this battle, the development of antibiotics was a game-changing turn. Unfortunately, more and more strains of pathogenic bacteria have become antibiotic resistant. One of this century's greatest medical challenges is the rapid emergence of multidrug-resistant pathogens. Discovery of new antimicrobial classes and alternative therapeutic options would be especially welcomed in this era. The fact that some bacterial infections might no longer be successfully treated with antibiotics, combined with an increasing population density and mobility, justifies the urgent demands for the development of novel treatments, of which immunotherapies are considered most promising. Immuno modulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases. Nowadays, treating emerging chronic and multidrug-resistant infectious, autoimmune and oncological diseases through reinforcement of the immune system became a major priority.

Keywords: Antibiotics, Antibodies, Autoimmune diseases, Bacteria, Bacterial infectivity, Cancer, Evasion immune system, Growth Factors, Host resistance, Host defences, Immune response, Immune system, Immunomodulators, Immuno

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therapy, Interferons, Interleukins, Multiresistant Infection, Passive Immuno therapy, Vaccines, Virulence.

1. BACTERIAL INFECTIVITY, IMMUNE RESPONSE AND MULTIRESISTANT INFECTIONS : NEW PERSPECTIVES

Bacterial infectivity results from a disturbance in the balance between bacterial virulence and host resistance. The "objective" of bacteria is to multiply rather than to cause disease; it is in the best interest of the bacteria not to kill the host. However, sometimes the story is not that simple.

1.1. Introduction

'good fences make good neighbours'

This section is about a fine tuned dance practiced over and over again through evolutionary times between a pathogen and its host, in a very Darwinistic way. This is the story of pathogens that are usually smaller than a human cell looking for resource-rich environment in which to survive and reproduce, and of their host fighting them with defense mechanisms and experience accumulated over hundred millions of years of evolution [1].

Infection is the invasion of the host by microorganisms, which then reproduce in close association with the host's tissues. Bacteria can cause a multitude of different infections, ranging in severity from unapparent to fulminating [2].

The ability of bacteria to cause disease reflects its relative pathogenicity. On this basis, bacteria can be organized into three major groups. Primary pathogens are considered to be probable agents of disease (*e.g.*, when the cause of diarrheal disease is identified by the laboratory isolation of *Campylobacter* spp. from feces). Opportunistic pathogens are those who colonize the body with no ill effect for most of the time but which can cause illness if the body's defenses are undermined. *Staphylococcus aureus*, a bacterium that colonizes human skin is also a common cause of pimples and can even cause food poisoning. *Escherichia coli* does no harm if it stays in the intestinal tract, but it can cause disease if it gains access to the urinary tract or the bloodstream. Finally, some bacteria, such as *Lactobacillus acidophilus* for man, are considered to be nonpathogens, because

they rarely or never cause disease in their host. Some nonpathogens are beneficial for the host and some have no effect. Nevertheless, their categorization as nonpathogens is in no way final, in part due to plasticity of bacteria. In fact, some bacteria previously considered to be nonpathogens are now known to cause disease.

Virulence is the measure of the pathogenicity of an organism. The degree of virulence is related directly to the ability of the organism to cause disease despite host resistance mechanisms [2]; it relies upon many variables such as the number of infecting bacteria, the route of entry into the body, innate and adaptive host defense mechanisms, and inherent virulence factors of the bacteria. Therefore, the determinants of virulence of a pathogen are any of its genetic or biochemical/structural features that facilitate its process to produce disease in an individual [2].

The relationship between a host and a pathogen is dynamic, and its outcome depends on the virulence of the bacteria and the relative degree of resistance or susceptibility of the host at that particular occasion.

Virulence can be measured experimentally by determining the number of bacteria required to cause animal death, illness, or lesions after the bacteria are administered by a designated route. Calculations of a lethal dose affecting 50 percent of a population of animals (LD_{50}) or an effective dose causing a disease symptom in 50 percent of a population of animals (ED_{50}) are useful in comparing the relative virulence of different bacteria [3].

Pathogenesis refers both to the mechanism of infection and to the mechanism by which disease develops. Here, we focus on the first aspect.

Fortunately for us, only an infinitesimal minority of bacteria are able to avoid the host defenses and eventually cause disease. In order to comprehend the mechanisms by which pathogenic bacteria cause disease, let us briefly mention some of the obstacles those small numbers of bacteria must overcome.

1.2. Host Resistance

The skin is the body's first defense against the microbial world and has several hidden weapons to be successful. Firstly, it forms a tight, impenetrable barrier of

CHAPTER 6

Perspectives on Natural Products

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Abstract: The emergence of antibiotic resistant strains along with the increasing public health concern regarding infectious diseases and hospital-acquired infections has led to the necessity of finding viable alternatives to antibiotics.

Nature has been a source of compounds with interesting medicinal properties for millennia. For many decades, natural products have been a wealthy source of antimicrobials and more recently academic drug discovery in this research area has been accentuated, though pharmaceutical industries devote fewer resources to antimicrobial drug discovery programs, in comparison with their investment several decades ago.

Though, initially, the developments in drug discovery were aimed towards synthetic chemical libraries, nowadays they are also greatly used in the research of bioactive natural products, in order to keep up with the developments in similar areas. Moreover, these developments allowed to overcome several problems associated with natural products drug discovery by using molecular techniques (i.e. genome mining) when organisms are not cultivable on the laboratorial environment.

In this chapter, various examples of natural products with antimicrobial properties from marine organisms and plants are referred, highlighting their important and promising results against pathogenic bacteria, fungi, viruses and protozoa.

Keywords: Antibacterial, Antibiotics, Antifungal, Antiprotozoal, Antiviral, Bacteria, Bioactive molecules, Drug discovery, Fungi, Infectious diseases, Marine organisms, Natural products, Pathogenic microorganisms, Plants, Secondary metabolite.

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1. NATURAL PRODUCTS AS ANTIBIOTIC ALTERNATIVES FOR THE NEW MILLENNIUM

Natural products are frequently referred to as secondary metabolites produced by living organisms and are composed of small molecules which lack an apparent function in growth, development, reproduction or survival processes on the producing organism. Instead, these secondary metabolites have attracted attention because of their biological effect on other organisms [1].

Initially regarded as waste products, natural compounds often present an ecological role in the regulation of the interactions between animals, plants and microorganisms, acting as defensive substances against predation and consumption, attractants or pheromones [2, 3].

Hence, natural products can be (i) entire organisms, either plant, animal or microorganisms, which have not been subjected to any processing besides its preservation; (ii) part of an organism, such as leaves of a plant or an animal organ; (iii) an extract of an organism or an exudate or (iv) pure compounds isolated from plants, animals or microorganisms [1].

Since ancient human history, natural products have been used for healing and relieving diseases. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is described in different manuals of phytotherapy; on the other hand, lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are reported as broad spectrum antimicrobial agents, in particular tea tree essential oil is used to treat acne and other skin infections [4].

Based on their use in traditional medicine, a large number of modern drugs have been derived from natural sources. A quarter to half of currently available drugs was originated from natural compounds [5]. Naturally occurring molecules serve as the source for the majority of the medicaments used in clinical settings and more than 50% of Food and Drug Administration (FDA)-approved drugs are either natural products or derivatives. Relatively to disease categories, approximately 68% of anti-infective (anti-bacterial, -fungal, -parasitic and -viral) were classified as naturally-derived or naturally-inspired, whereas in cancer

treatment these numbers rise above 79% [6].

Their structural diversity along with their unique and complex structures has long been appreciated, being very important scaffolds in drug discovery research. Moreover, the fact that most natural resources remain unknown and undiscovered is remarkable. Only 6% of the 300,000 species of higher terrestrial plants have been investigated in the pharmacological and chemical area. The same happens in the marine environment, where most areas remain unexplored [2].

Since the late 1980s, the average number of new chemical entities has been decreasing from over 60 small molecules per year to only 23, from 2001 to 2010 [6]. The lack of interest in natural products by major pharmaceutical companies in favor of new chemical techniques was one of the factors responsible for this declining in the research on natural products [7].

Nevertheless, for years to come, natural products will still be relevant in new drug research, due to their inherent unparalleled chemical structural diversity and qualifications regarding "(i) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semisynthetic and total synthetic modification; (ii) the number of diseases which can be treated or prevented by these substances; and (iii) their frequency of use" [8]. Additionally, these compounds present a tendency to interact with biological targets, meaning they are suitable lead structures.

2. SEARCHING FOR NATURAL PRODUCTS: THE IMPORTANCE AND EVOLVING ROLE OF NATURAL PRODUCTS IN DRUG DISCOVERY

Natural compounds have made and continue to make a unique contribution for the discovery and development of effective drugs to be used for the treatment of various human diseases. They can be applied as new drugs to be readily used in an unmodified state (*e.g.* vincristine from *Catharanthus roseus*), as chemical scaffolds used to synthesize other complex molecules (*e.g.* diosgenin from *Dioscorea floribunda* for the synthesis of oral contraceptives) or as indicators of new ways of pharmacological action in order to synthesize new analogs (*e.g.* synthetic analogs of penicillin from *Penicillium notatum*) [1].

CHAPTER 7

Bacteriocins

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Abstract: Bacteriocins are antimicrobial peptides produced by numerous bacteria, which may present narrow or broad host range. These ribosomally synthesized antimicrobial peptides are considered a successful strategy in maintaining equilibrium within a bacterial ecosystem. These compounds kill other bacteria by several mechanisms, including the modification of membrane permeability and depolarization of membrane ion gradients, the degradation of nucleic acids or cell walls. In literature, the term bacteriocin is usually restricted to peptides produced by Gram-positive bacteria, while in Gram-negative bacteria, mainly enterobacteria, the toxins are called either colicins (*i.e.* antibiotic proteins targeting *Escherichia coli*) or microcins. Many bacteriocins are produced by food-grade lactic acid bacteria, a phenomenon which offers the possibility for preventing the development of specific bacterial species in food. This can be particularly useful in preservation or food safety applications, but also has implications for the development of desirable flora in fermented food. In this sense, bacteriocins can be used to confer a protection and at the same time help processors extend shelf-life after product manufacture

Keywords: Anticompetitors, Antimicrobial-peptides, Archeosins, Bacteriocins, Bacteriolytic enzymes, Biopreservation, BLIS, Bovamine, Colicins, Detection, Enterocin, Food-preservatives, Genetic-regulation, Halocin, Hemolysin, Immunity, LAB, Lacticin, Lantibiotics, Lysostaphin, Mersacidin, Microcins, Mode of action, Nisaplin, Nisin, Novasin, Pediocin, Pre-peptide, Secretion, Spot-on-lawn, Streptolysin, Toxin.

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1. INTRODUCTION

The use of antimicrobial peptides is one of the most widespread strategies against microbes. These substances are produced from insects to plants and even humans. Antimicrobial peptides can be fast acting and usually present broad-spectrum activity, which diminishes the possibility of resistance developing in target species. Production of these peptides can also be found in Bacteria and Archaea. Bacteria produce an enormous diversity of antimicrobial agents including antibiotics, metabolic byproducts, such as lactic acids produced by lactic acid bacteria (LAB), lytic agents such as lysozymes and numerous types of protein exotoxins, such as bacteriocins.

Bacteriocins are ribosomally synthesized, small, heat-stable peptides that upon release extracellularly can interfere with the growth of other bacteria. Bacteriocins can present a narrow (same species) or broad (across genera) target spectrum. Producer-organisms are immune to their own bacteriocins through the production of specific immunity proteins [1 - 3].

The term *bacteriocins* was first defined by Tagg *et al.* (1976) [4] as proteinaceous compounds able to kill closely related bacteria. While true for the majority of bacteriocins, for some, it could also be true for bacteria confined to the same ecological niche [5]. In 1877, Pasteur and Joubert reported an inhibitory activity against the anthrax bacilli associated with bacteria isolated from urine, probably *Escherichia coli*. The first studies on bacteriocins were performed by Gratia and Fredericq (1925) on Gram-negative bacteria, especially colicins produced by *E. coli* [6]. By this time, bacteriocins were defined based on colicins properties such as being plasmid-encoded, large domain-structured proteins, having bactericidal activity through specific receptors and being lethal SOS-inducible biosynthesis (Fredericq, 1946) [7]. However, few Gram-positive bacteriocins fit this classical colicin definition. The main differences are further explored in this chapter (see “Diversity of Bacteriocins in Gram-positive Bacteria”). Shortly, they include relatively broad activity spectra; producer cell self-protection (immunity); and absence of SOS- inducibility. During the last decades, studies in Gram-positive bacteria bacteriocins have dominated bacteriocin-related literature, with special focus on Lactic Acid Bacteria (LAB), driven by commercial interests [2].

There are two main features that distinguish bacteriocins from antibiotics: (i) bacteriocins are ribosomally synthesized, while antibiotics are not; and (ii) bacteriocins have a relatively narrow killing spectrum, while most antibiotics present broad killing ranges. The bacteriocin family includes a number of proteins that vary in size, microbial target, mode of action, release and immunity mechanisms, and can be divided into two main groups, those produced by Gram-negative or by Gram-positive bacteria.

Bacteriocins are found in almost every bacterial species examined to date, and within a species tens or even hundreds of different kinds of bacteriocins are present. According to Klaenhammer, 99% of all bacteria may produce at least one bacteriocin and the only reason we have not found more is that we haven't looked for them [8]. This diversity and abundance of bacteriocins suggest an important role for these antimicrobials agents. These toxins have been found in all major lineages of Bacteria, and more recently, have been described as universally produced by some members of the Archaea (see "Bacteriocins from Archaea"). In this chapter we summarize literature that has helped unravel the mysteries of bacteriocins since their discovery; explore diversity, genetic regulation and mode of action and applications in food safety and human and livestock health.

2. BACTERIOCINS IN GRAM-NEGATIVE BACTERIA

The first description of bacteriocin-mediated inhibition was reported in 1925 by Gratia and Fredericq in antagonistic strains of *E. coli* [6]. These compounds were originally designated as colicins to reflect the producing organism. Bacteriocins produced by Gram-negative bacteria are diverse. Just in *E.coli* over 30 different bacteriocin forms have been identified.

Levels of bacteriocin production in Gram-negative bacteria, such as *E. coli*, *Salmonella enterica*, *Hafnia alvei*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* range from 3 to 26% of environmental isolates. Colicins, produced by *E. coli*, were found in 30–50% of human strains. Much higher production levels have been found in some other bacteria, such as *Pseudomonas aeruginosa*, in which 90% or more of both environmental and clinical isolates produce bacteriocins [9].

CHAPTER 8

Biocides – A Reasonable Alternative to Prevent and Control Microorganisms?

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Abstract: Acute infections caused by pathogenic microorganisms have been studied extensively for over 100 years. Infections that killed millions of people in the past are now ever more successfully controlled due to the development and use of antimicrobial compounds. Biocides, in particular, have been used in various forms for many centuries to control the dissemination of microorganisms and represent a wider group of substances with a key role in the medical and food industry. This chapter presents the main biocidal classes and mechanisms of action, including their respective spectrums of activity and use in several fields. Factors influencing the effectiveness of biocides are addressed as well as biocide resistance and the link with antibiotic resistance. Several mechanisms of biocide resistance are presented on a molecular level with a particular focus on biofilm resistance and its formation in bacterial populations. This chapter concludes with an introduction to ECHA, the European Chemicals Agency and their recently approved regulatory framework for biocide products in Europe.

Keywords: Antimicrobial Resistance, Biocides, Biofilm, Concentration, Contact Time, Cross-Resistance, Disinfection, ECHA, Effectiveness, European Union, Exopolymeric products, Legislation, Microorganisms, pH, Quaternary ammonium compounds, REACH, Regulation, *Salmonella*, *Staphylococcus aureus*, Temperature.

1. INTRODUCTION

Antimicrobial resistance in bacterial pathogens and other microorganisms is disseminated worldwide leading to therapeutical failures in both human and animal diseases. This situation poses an emergent challenge in research, aiming at

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the identification of new approaches to control multidrug resistant pathogens by discovering new compounds or increasing the effectiveness of pre-existing ones such as antibiotics or biocides [1, 2].

The biocides have been used in various forms for centuries. These compounds are chemical substances with the ability to impair, render harmless, or apply a controlling effect on any microorganism by chemical or biological means. Biocides represent a wider group of substances with a key role in pharmaceutical and medical industry [3]. They are also commonly used in many other fields like agriculture or even forestry [4 - 6].

In the past, several references were made regarding the use of these chemical substances: empirical approaches of copper and silver vessels for storing potable water, vinegar and honey for disinfecting wounds or even balsams as preservatives in mummification. Later, reports were made about the use of iodine as a wound disinfectant, of chlorine water in obstetrics and the claimed sporicidal activity of mercuric chloride [7, 8].

In the beginning of the twentieth century, biocides such as chlorine agents and some quaternary ammonium compounds (QACs) were presented [9]. In 1945, several groups of biocides were commonly used, in particular: phenolics, organomercurials, chlorine agents, iodine, alcohols, formaldehyde, hydrogen peroxide and silver compounds [8].

Nowadays, probably one of the most important agents introduced, especially in the medical industry, are the biguanides, namely chlorhexidine. This compound is a cationic polybiguanide with antifungal and bactericidal properties [10]. Its importance was soon recognized in 1979, when chlorhexidine was considered by the World Health Organization in the List of Essential Medicines as a critical compound needed in a basic health system [5, 11].

Today, the interest and need for biocides is not diminished. On the contrary, its use has expanded and it is completely widespread in all types of industries. In 2006, the global biocide demand was estimated at €10-11 billion with a growth of 4-5% per annum for the previous 15 years, and the market expansion is predicted to continue. Despite the increased demand in biocides, application and selection

of biocides has become more restricted in the last three decades. These restrictions were applied in response of two main concerns: product toxicity and environmental impact [12].

Regardless market constraints, biocides are promising compounds being effective against virulent and antimicrobial resistant bacteria such as *Escherichia coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug resistant *Salmonella* spp. [13 - 15]. These substances can also be active against fungi [16]. Biocides also show an efficacy against bacterial biofilms, one of the major reasons for chronic relapsing infections, impairing biofilm formation [17]. These compounds show a great ability to deal with resistant microorganisms, and the emerging resistance against different types of biocides has only been studied and characterized recently [18, 19].

New antimicrobial therapeutic options to treat multi-resistant bacteria are progressively decreasing and biocides may be a promising alternative to impair their dissemination [20, 21]. The purpose of this chapter is to review biocides classes, mechanism of action and effectiveness with a particular focus on the mechanisms of biocide resistance, including biofilm.

2. BIOCIDES CLASSES

“Biocide” describes a chemical agent, natural or synthetic, that inactivates microorganisms and usually has a broad spectrum [22]. The antimicrobial activity of these compounds can range from inhibiting the target growth, in this case we use the term “-static,” to kill the target, the term “-cidal”. Biocides include antiseptics, disinfectants and preservatives. Antiseptics are products largely used in the medical field that inhibit the growth or the development of microorganisms in living tissue, not necessarily killing, such as oral hygiene and wounds cleaning. Disinfectants are generally used on inanimate objects or surfaces because they can injure the tissues, inactivate most microorganisms but not commonly spores, and are used in many applications in daily life.

Preservatives act to prevent the microbe’s multiplication in formulated products, including foods and pharma-ceuticals [22 - 24].

CHAPTER 9

Novel Therapeutic Strategies in Veterinary Medicine

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Abstract: The use of antibiotics in animal production has led to the emergence of antibiotic resistant microorganisms with severe consequences in animal and human health. To counterbalance and substitute the use of antibiotics as antimicrobial agents, a comprehensive effort has been directed towards the development of new therapeutic strategies and Veterinary Medicine offers an appealing field for the implementation of such an effort. Probiotics and bacteriophages are two of the alternatives discussed.

Keywords: Antibiotic resistance bacteria, Bacteriophages, Food-borne pathogens, Microbiome, Phage therapy, Probiotics.

1. INTRODUCTION

The emergence of antibiotic resistant microorganisms, caused by an improper use of antibiotics is a major public health concern, pressing towards the development of new therapeutic strategies, which may counterbalance or substitute the use of antibiotics as antimicrobial agents.

Veterinary Medicine provides an appealing field for the use and development of novel therapeutic strategies due to the close link and sanitary impact of animal health in public health. Intensive animal production in highly stressful conditions increases the animals' susceptibility to microbiota imbalance increasing the risk of colonization by pathogenic microorganisms. In small animal practice there is a growing awareness regarding the development of antibiotic resistance

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mechanisms by potential zoonotic microorganisms.

This chapter focuses on two strategies: probiotics and bacteriophage therapy and their scope in Veterinary Medicine. Probiotics interact with the host microbiota, contributing to a higher health performance and disease control, both in production animals, and in small animal's medicine. Bacteriophages are bacteria virus, used for the control of bacteria pathogens. Both are used as biocontrol agents, although with different applications and scope.

1.1. Probiotics

It was Ilya Ilyich Metchnikoff (1845–1916) who introduced the concept that probiotics contributed positively for the enhancement of health [1]. Since then, the scientific basis of this concept have been thoroughly studied and the basic mechanisms by which probiotics contribute to the host homeostasis are beginning to be unraveled.

Probiotics are defined as "live microorganisms, when administered in adequate amounts confer a health benefit on the host" [2]. They provide a mean to restore the host microbiota, lost by death or excretion. Presently probiotics include viable bacteria, yeast and fungi, without adverse effects, contributing to the host health [3].

The mechanisms by which probiotics restore intestinal balance and subsequently prevent disease include: (i) stimulation of the host immune response; (ii) production of antimicrobial products; (iii) competitive exclusion of pathogenic microorganisms; and (iv) blockage of microbial toxins, complementing and/or substituting the role of the commensal microbiota [4, 5]. These actions complement each other, enhancing microbiota diversity, preventing intestinal colonization by pathogenic microorganisms, contributing to infection quell, and the recovery of homeostasis between the microbiome and the host [6].

Each organism has its own microbiome with specific microbial species and strains and critical differences between domestic animals and human are easily identified, due to the high physiological diversity between animal species. The present knowledge supporting the use of probiotics in Human and Veterinary Medicine is

mostly based on animal models and *in vitro* studies, although a substantial effort is being made to characterize which microbial species and strains should be used in each animal species and in which situation [6].

In 1989, Fuller recorded that a good probiotic should be: i) a strain capable of exerting a beneficial effect on the host animal, promoting increased growth or resistance to disease; ii) non-pathogenic and non-toxic; iii) present as viable cells, preferably in large numbers; iv) capable of surviving and metabolizing in the gut environment; and v) stable and capable of remaining viable for long periods under storage and field conditions [7]. Additionally probiotics should not acquire antibiotic resistance genes and should not recombine with pathogenic microorganisms [8]. This definition is still updated, but nevertheless open to the inclusion of new features according to recently gathered information on probiotics action.

In Veterinary Medicine, probiotics are mainly intestinal probiotics and are used as growth promotors in farm animals, improving food digestibility and also to stabilize gastro-intestinal microbiota and prevent intestinal infections [9]. In small animals, in gastroenterology due to their action as microbiota stabilizers.

probiotics improve nutrition and are also used

1.2. Chicken and Turkeys

In healthy poultry, a balance amid the intestinal microbiome persists. Whenever there's a change in environmental conditions leading to stress, a shift between microbial populations occurs, with a decrease in favorable species, mainly lactobacilli, and an increase in detrimental microbial species, with a direct consequence on poultry health and eventually leading to disease. Due to husbandry methods, young chickens are deprived from their parents being unable to receive gut flora from their mothers' feces, making probiotic supplementation in these animals particularly beneficial, due to its direct effect on the gut microbiome composition and balance [10].

In broiler nutrition, the most current microbial species used as probiotics are highly diversified and include *Lactobacillus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Pediococcus* and *Bifidobacterium* genus, *Escherichia coli*, yeasts

Summary

Frontiers in Antimicrobial Agents –The Challenging of Antibiotic Resistance in the Development of New Therapeutics

The prevalence of drug-resistant microorganisms has increased worldwide [1]. However, in the last years, few novel entities to fight drug resistant microorganisms have entered the clinic. The antibacterial pipeline is scarce because of the costs associated with the clinical trials and licensing of the antibiotics [2]. The emergence of multiresistant strains and the temporal and geographical variations in resistance patterns suggest the need to look for alternatives to the classic antimicrobial therapy. The development of novel entities to control the dissemination of drug resistant microorganisms would decrease the morbidity and mortality caused by these disease causing agents, and consequently the economic burden associated with health treatments.

The present book, entitled "Frontiers in Antimicrobial Agents – The challenging of antibiotic resistance in the development of new therapeutics", gathers a wide range of topics on the subject and includes several chapters with original material. Authors and co-authors represent a multidisciplinary team that includes scientists with a vast experience in the area, from different universities and research institutions. Topics addressed include: Phage therapy, Antimicrobial Peptides (AMPs), Probiotics, Immunotherapy, Natural Compounds, Bacteriocins, Biocides, and Novel Therapeutic Strategies in Veterinary Medicine. The book describes innovative alternatives to the classical antimicrobial therapeutics, based on cutting edge research, representing a huge advance to science and technology. Some of these approaches are considered extremely valuable in the nearly future due to their low toxicity, capacity for large scale production, and most importantly their low probability to generate resistance.

The book will be extremely valuable for all those who are interested in antibiotic resistance, and in strategies to the classic antibiotherapy being a significant advance to the state-of-the-art in this regard. The variety of areas focused will attract a broad range of readers, including researchers, clinicians, professors and students. It will also enlighten readers regarding different approaches to control the dissemination of drug resistant microorganisms.

[1]. World Health Organization (WHO). Antimicrobial Resistance - Global Report on

SUBJECT INDEX

Manuela Oliveira and Isa Serrano (Eds)

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Manuela Oliveira has a degree in Biology and Microbial Genetics from the Faculty of Sciences, University of Lisbon, since October 1996; a Master in Food Science and Technology from the Technical University of Lisbon since July 2000; a PhD in Animal Science and Technology from the Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) since September 2005; and a Master in Veterinary Medicine by FMV / UL since July 2013. In addition to her vast research experience in veterinary bacteriology, antimicrobial resistance, biofilms and virulence factors, demonstrated by authoring 30 articles in international scientific journals and 10 book chapters, amongst other publications, she has experience in bacteriological diagnosis, collaborating in routine analyzes performed in the Bacteriology Laboratory of FMV/UL since July 2005. She is currently an Invited Assistant Professor at FMV/UL, teaching Microbiology and Food Microbiology.

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Isa Serrano is graduated in Biology from the Faculty of Sciences, University of Lisbon since 2000, and concluded the MSc Degree in Molecular Microbiology from the Aveiro University in 2003 and the PhD in Health Sciences, specialization in Microbiology, from the Faculty of Medicine, University of Lisbon (FMUL) in 2008.

During her 13 years of research experience she worked mainly at Institute of Molecular Medicine where she carried out the PhD and Post-doc. She is a specialized researcher in microbiology, epidemiology of Streptococcus pneumonia, antimicrobials resistance, clone spreading, and virulence traits. She has also a long experience in models of cell barriers and in drugs tested for their cell permeability, and analgesic efficacy. The outcome of her work is patent in 16 publications in international peer-review journals, and several communications. She supervised several students, and was an invited Assistant Professor of Parasitology at FMUL for three years. She is currently a member of the Faculty of Veterinary Medicine, University of Lisbon.