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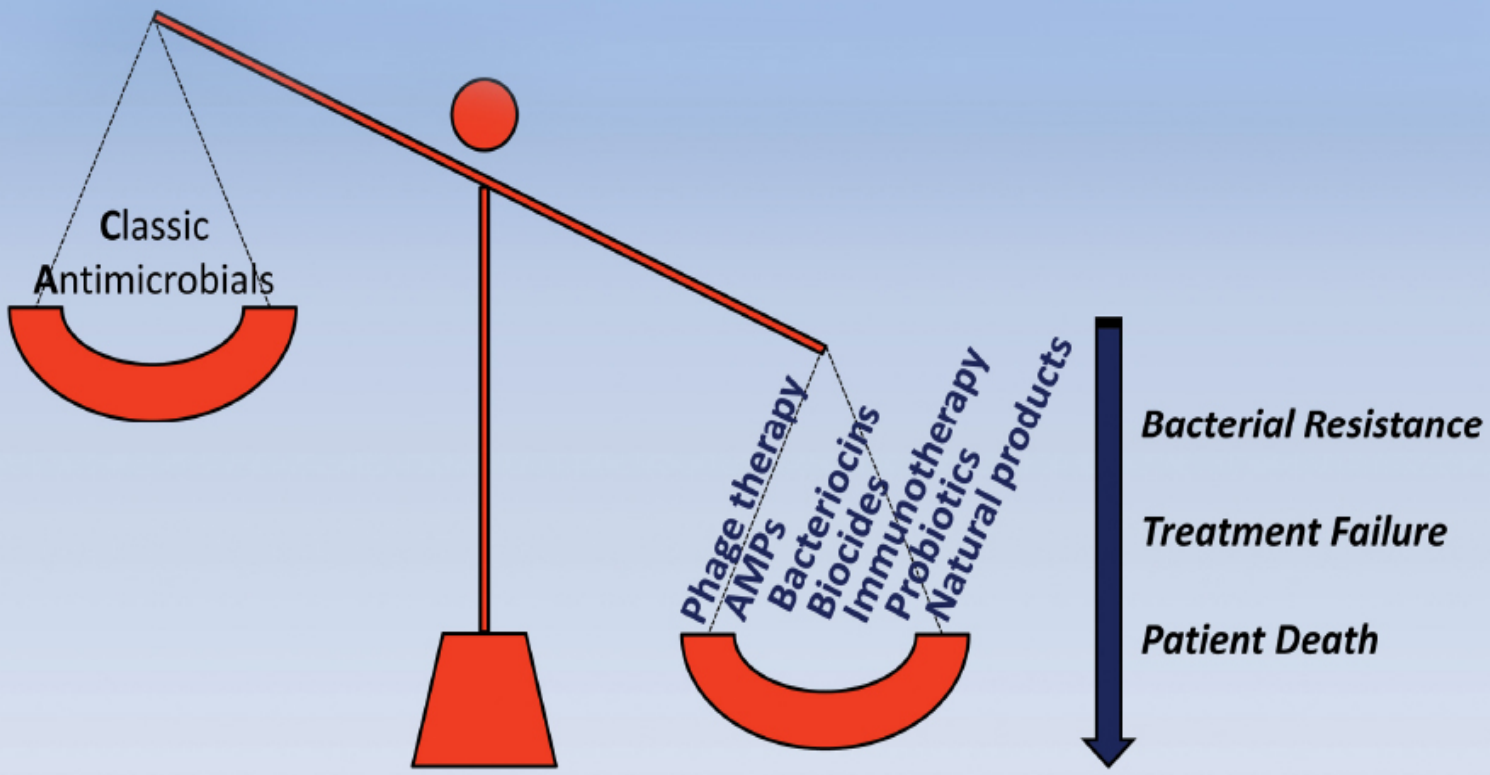
FRONTIERS IN ANTIMICROBIAL AGENTS

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

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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