Strategies for Vaccination and Control of Apicomplexan Protozoan Parasites

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Over the past several decades a great deal of effort has been invested in developing new control and vaccination strategies for apicomplexan protozoan parasites. These organisms are the cause of some of the most significant and harmful diseases in both humans and animals worldwide and include as examples; *Plasmodium* (malaria) and *Cryptosporidium* in humans, *Toxoplasma* in humans and animals, *Babesia* and *Neospora* in cattle, and *Eimeria* the cause of coccidiosis in animals (poultry, sheep, cattle, etc.). In spite of a great deal of progress made in understanding at the molecular level how these organisms invade, survive and transmit in their respective hosts, there has been a paucity of new vaccines commercially developed against these parasitic protozoa over the past few decades. In this chapter, we will discuss the types of strategies being developed to help control these parasites, which includes the development of live and subunit (both native and recombinant DNA based) vaccines, the search for and use of new or existing drugs (such as artemisinin combination therapy against malaria), as well as simpler management & hygiene strategies that can be employed to help alleviate the burden of parasitic diseases.

Keywords Apicomplexan parasites; vaccines; drug targets; ecoimmunology; immunotolerance

1. Introduction

As a result of the development of both recombinant DNA and hybridoma technologies in the early 1970s, it was believed that within 5-10 years new vaccines would be developed against malaria as well as several of the other major parasitic diseases affecting human and animal health. Unfortunately, those hopes have yet to be realized and although excellent progress has been made in unravelling some of the mysteries associated with parasite growth and developmental biology, little has been accomplished in the actual control of these devastating pathogens. Indeed, due to the development of drug resistance by the malaria parasite, the number of people infected in the world had actually grown to 244 million in 2005, but then decreased to 225 million in 2009 mainly due to the use of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS). Similarly, the number of deaths from malaria also increased during the 1970s-1990s, and then decreased from 985,000 per anum in 2000, to 781,000 in 2009. However, in some countries in Africa and elsewhere there has continued to be a marked increase in malaria morbidity and mortality over the past 5 years, which has been disappointing for the WHO and others involved in malaria control.

Much research has been devoted to the developmental biology of these parasites (i.e. their life cycle and mechanisms of switching from one developmental stage to another) and how they interact with the host in order to try and discern weaknesses in the parasite‘s armour. Clearly, parasitic protozoan parasites have come through several million years of coevolution with their hosts and have learned to invade and survive in very hostile environments. For example, the *Toxoplasma* parasite lives within the cell (macrophage) whose sole purpose is to destroy incoming foreign pathogens. *Plasmodium* and *Babesia* parasites have learned to invade host red blood cells or reticulocytes without lysing them using specific subcellular organelles (rhoptries and micronemes) present in the merozoite stage of development, and in that manner evade the host immune system. *Cryptosporidium* and *Eimeria* parasites reside within the intestinal epithelial cell and develop to an oocyst stage after which they are excreted in the feces. In this manner the parasite can survive for months or even years under harsh environmental conditions, enabling them to infect other host animals or humans. These apicomplexan parasites have also learned to move from host to host through arthropod vectors where they are transmitted to entire human or animal populations. In this chapter we will explore these various developmental and host/vector evasion strategies and discuss ways in which to inhibit or even prevent their growth and development.

It is clear that in order to control apicomplexan parasitic protozoa we must be able to rethink many of the current strategies that have been taken up in this battle against these pathogens. As mentioned above, apicomplexan parasites are very capable of evading host immune responses and surviving in hostile environments. In addition, they have learned to deal with antimicrobials produced to destroy them, developing resistance against a whole variety of drugs and toxic compounds. So where does the achilles heel of these parasites lie? This question will be discussed in the context of our current knowledge of host immune evasion and basic parasite biology.

Finally, in this chapter we will look at other potential control strategies involving improvements in how we manage the environment in which these parasites live and are transmitted (i.e. the ecosystem & ecoimmunology). This includes an understanding of the effect of host nutrition, environmental factors as well as infection with multiple pathogens on the immune response. Finally, we will discuss the question of whether or not we can really defeat these parasites or perhaps at least in certain cases we should be looking to develop a strategy of inducing immunotolerance to reduce the

harmful effects of the infection. All in all, it is clear that a great deal of new and original research must be designed and carried out in order to help alleviate the terrible health burden and economic cost of these parasitic diseases.

2. Parasite developmental biology

Apicomplexan protozoan parasites undergo their life cycle in various tissues and cell types within the host animal or human. *Plasmodium*, the parasite which causes malaria in humans and animals, first infects the liver cells in the form of sporozoites where they undergo the extraerythrocytic, asexual life cycle. From there, daughter merozoites are released into the blood stream where they go on to infect red blood cells relying on organelles contained in the apical end of the parasite (rhoptries and micronemes) to invade the cell without rupturing it. They go on to form a parasitophorous vacuole where they can reside safe from the host immune response. Within this vacuole the parasite divides asexually producing new daughter cells (merozoites) which are released when the cell bursts open. After several such replication cycles during which the pathogenesis of malaria is manifested (fever and chills, anemia, cerebral malaria, etc.), the parasite once again invades red blood cells and commences the sexual cycle of development forming male microgametes and female macrogametes. These are taken up by a mosquito in a blood meal and in the midgut they undergo fertilization, zygote formation and development into ookinetes, which penetrate the gut wall and develop into single walled oocysts. The parasite undergoes mitotic replication in the oocyst forming numerous sporozoites that exit the oocyst and reach the salivary gland where they are injected into the next human host during the blood meal.

In the case of *Eimeria* and *Cryptosporidium* similar, less complex, developmental life cycles occur in the epithelial cells of the host intestinal mucosa. Here these parasites undergo both the asexual and sexual stages of their development life cycle, and over a period of 4-8 days form infectious oocysts which are secreted in the feces (in the case of *Cryptosporidium*, oocyst shedding can continue for several weeks post infection due to autoreinfection). These highly resistant and stable oocysts are then picked up by other animals or humans due to their presence in either litter, contaminated food, or water supplies. Due to their rapid replication and exponential increase in numbers (for each oocyst ingested up to 1,000,000 new ones are formed and secreted), the parasite is transmitted through the population where it can cause severe disease due to the rupture of host cells, secretion of toxic substances, as well as by inducing immunopathology. Indeed, it is due to the intense rearing practices of domestic animals, poor hygiene practices, and in the case of *Cryptosporidium*, difficulty to prevent the introduction of these parasites into drinking water due to their resistance against most disinfectants and very small size, that these parasites have caused such widespread damage.

Toxoplasma is an example of a parasite where in its definitive host, the cat, it undergoes its developmental cycle in epithelial cells of the intestinal tract, producing oocysts that are secreted in the feces into the environment. Once the oocysts are ingested by an intermediate host such as rodents, cattle or sheep, the sporozoite penetrates the intestine, is disseminated in the host and finally develops into tissue cysts where it can survive in undercooked meat until ingested by humans, cats or other animals. In the human or animal intermediate host, the parasite will develop in tissues and the bloodstream as tachyzoites, during which time the parasite invades macrophages and survives in phagosomes by preventing fusion with lysosomes as well as increasing the longevity of its host cell. After induction of a protective immune response by the host, they form bradyzoites that develop into cysts in the musculature, heart and brain. There they can reside for the entire lifetime of the host until activated either due to immunosuppression by viruses such as HIV or immunosuppresive drugs, forming tachyzoites that can directly damage host cells as well as induce an immunopathological response. This can lead to severe problems in the brain (encephalitis), eyes (chorioretinitis) as well as problems in the inner ear, heart and liver.

Over the past several years, live vaccines have been developed for some of these parasitic diseases. In the case of Coccidiosis, these vaccines were developed based on the finding that a single infection with an *Eimeria* species induces a very high level of immunity against reinfection against the homologous species [1]. As one example of a live coccidiosis vaccine, Paracox® was developed in which attenuated lines for each of the 7 species of *Eimeria* that cause the disease were combined and delivered to chickens via the drinking water [2]. Good results were obtained in that chickens that were vaccinated at 1 day of age with Paracox® were solidly immune against infection and disease by 2-3 weeks of age.

A similar approach has been taken for malaria where it has been shown that live infection can induce good immunity against reinfection. Researchers in Australia and the USA have begun the development of a live vaccine using parasites produced in tissue culture [3]. However, it is difficult to produce live malaria parasites in vitro at a low cost. In addition, storage, transport and use of such a vaccine in endemic countries would be very difficult to implement. Furthermore, malaria parasites have been shown to undergo antigenic variation so that it may be necessary to combine a variety of parasite strains in any live vaccine. In spite of these problems, it is hoped that a program to vaccinate against malaria in endemic countries using a live vaccine may be achievable in the future.

The fact that live vaccines can induce solid immunity show that there are chinks in the armour of these parasites that can be exploited to produce subunit or recombinant DNA based vaccines to inhibit or even prevent their growth and development. In this regard, one possible approach is to try and induce an immune response to prevent parasite invasion of the host cell by sporozoites, tachyzoites and/or merozoites. A great deal of information is available on the molecular composition of the organelles involved in invasion; the rhoptries, micronemes and dense granules. Many of these

proteins are well conserved between apicomplexan species and even genera, and several have been tested (either as a native or recombinant antigen or as a DNA based formulation) as vaccine candidates against a variety of apicomplexan parasites [4,5]. However, either partial or no significant levels of protection was observed in those studies. It is currently believed that by improving the methods of antigen presentation and delivery, better results with higher levels of protection can be obtained.

Another approach to vaccination against these parasites is to try and inhibit cyst/oocyst wall formation thereby reducing or blocking parasite transmission. In this regard, a subunit vaccine using purified antigens from the gametocyte and ookinete stages of development of *Plasmodium gallinaceum and P. falciparum* were tested to see if they can reduce the number of oocysts in mosquitos infected with blood from vaccinated birds or humans [6,7]. The results showed that a very high level of inhibition can be achieved reaching over 90% reduction of transmission. In *Eimeria*, vaccination of laying hens with gametocyte antigens or by live infection to induce a high level of maternal IgY antibody in their eggs, greatly reduced oocyst production in offspring chicks (by up to 80% using purified gametocyte glycoproteins to vaccinate the hens). This finding led to the production of a subunit vaccine containing 3 purified, native gametocyte antigens (CoxAbic®), which has already been tested and proven to be effective in controlling Coccidiosis in millions of broiler chickens around the world [8]. It was hoped that this approach could also be used to control diseases caused by other apicomplexan parasites in humans. However, the use of transmission blocking immunity in the control of *Plasmodium*, *Toxoplasma* or *Cyptosporidium* is much more difficult since it requires the induction of an antibody response that remains high for decades, while a chicken is only raised for 5-8 weeks of age before it is slaughtered for meat production. In addition, for malaria control a transmission blocking approach would require a great deal of eduction to convince people to participate in such a program in which no direct benefits are felt by the vaccinated individual. Finally, for Toxoplasma cats would need to be vaccinated to block parasite transmission.

3. Use of drugs in the control of apicomplexan parasites

Over the past 70 years, antimicrobial/antibiotic drugs have been developed to control diseases caused by apicomplexan parasites. In the case of malaria, in the late 1940s chloroquine was put into use and was extremely effective in combating this parasite. However, within 10 years of its use drug resistance appeared in Africa which spread worldwide. It was subsequently found that this resistance is based on point mutations occuring in key transport proteins (*pfcrt* and *pfmdr 1*), demonstrating the ability of the parasite to undergo mutations enabling its survival [9]. Similarly, the *Eimeria* parasite has become resistant against a multitude of anticoccidial drugs including potent ionophores, leading to the need for rotation and shuttle programs to reduce the build up of resistant parasites in the litter [10].

More recently artimisinin and artimisinin related compounds have been developed and used for the treatment of malaria, however, the WHO have already recommended that drug combination therapy be implemented to reduce the build up of drug resistance by the parasite. Once again mutations in transport proteins have accounted for the resistance phenotype [11]. New artimisimin related drugs and drug combinations are still being developed, however, only through strategic and limited use of these compounds will their effectiveness be preserved [12]. This is of course not only true for the malaria parasite, but for all parasites where new drugs are being developed. This coupled with the unwillingness of the pharmaceutical industry to invest large sums of money into developing new compounds, is leading to a situation in which we will have no effective means of treating malaria and other parasitic diseases.

4. Rethinking current strategies

As was mentioned above, apicomplexan parasites have been around for millions of years and have learned to grow and survive within a whole variety of hosts and environments. The strategies we have developed thus far to try and control them have largely been based on concepts that do not take into account the fundamental ability of these organisms to adapt, mutate and evade host immunity or environmental stresses. For example, parasite invasion is an attractive target to try and inhibit development, however, the organelles and proteins produced are well recognised by the host immune system, yet the parasite can still evade such an immune response.

Recent studies have indicated that it is not only a matter of the mounting of an immune response, but making sure it is of the right type and specificity. With regards to the cellular arm of the immune response, it has been found that the type of T cells and effector molecules and cytokines elicited can play a key role in determining the outcome of the infection [13]. This includes a proper balance between activation of 2 types of T helper cells (Th1 and Th2). Indeed, a given pathogen will employ a strategy to induce an immune response that benefits its survival such as by excreting substances that can inhibit the immune system, that activate the appropriate Th1 or Th2 cells for its own survival, as well as molecules that act to overstimulate the host immune system diverting it from the appropriate response. In the case of parasites such as *Eimeria* and *Cryptosporidium*, it was found that the host immune response can either play a key role in exacerbating intestinal lesions or inhibiting them depending on the type of immune response and host genetic background [14]. In addition, when multiple pathogens are present in the same host, the immune response becomes highly complex and unpredictable and may act to worsen one type of infection while preventing another [15].

A great deal of work using animal model systems with mixed infections have been performed, where it was found that the establishment and intensity of infection was effected by the combination of pathogens used (bacteria, virus, helminth or protozoan parasite), the strain of parasite being tested (highly virulent, mild, etc.), the host genetic background as well as the order in which the infectious organisms were administered.

So where does the achilles heel of these parasites lie? One approach already mentioned above is in the prevention or inhibition of cyst/oocyst wall formation. One thing is for certain, if an apicomplexan parasite cannot form its cyst/oocyst wall, it will not survive in the environment and be transmitted. A great deal of progress has been made in elucidating the molecular compostion and architecture of the oocyst wall of *Eimeria* and other apicomplexan parasites [15]. It is hoped that apart from immunological approaches to prevent oocyst formation, new drug targets can be discovered that may act to completely disrupt parasite developmental biology. Recent results showing artimisinin can prevent oocyst formation in *Eimeria* is very encouraging [16], and hopefully new drugs that are extremely potent in preventing cyst wall formation will be discovered and utilized.

Another potential strategy is by focusing on the pathogenicity of these parasites rather than trying to prevent their growth & development. For example, if the deleterious effect of *Cryptosporidium* is at least partly due to the toxin it secretes, then by either producing antibodies against these toxins or inhibitory drugs designed to block the toxic effect, then a new approach to controlling the severe diarrhea caused by this parasite may be accomplished [17]. In the case of *Plasmodium falciparum*, a great deal of the pathogenicity is due to its ability to attach to host cells and sequester itself away from the host immune system (the parasite modifies the erythrocyte surface membrane to bind to capillaries in the brain and thereby remove the infected host cell from the circulation). The sequestered parasites can then act to either directly block circulation in these capillaries, or to elicit an immunopathological response by host lymphocytes and macrophages leading to inflammation [18]. This process leads to the disease state known as cerebral malaria, which in severe cases can lead to coma and death. A vaccine that would induce antibodies that can block the binding of the parasitised red cell to the host cell membrane, or through the use of inhibitory peptides, etc., would alleviate this blockage in brain circulation and prevent the disease from occurring.

5. The role of management practices and the environment in parasite control.

Until vaccines or new effective drugs against most of these pathogenic protozoan parasites are developed, it is necessary to try and reduce infectivity and transmission through good management practices and improvements in environmental control. For example, simply by using insecticide impregnated bed nets and indoor residual spraying has led to a dramatic decrease in malaria transmission and a reduction in morbidity and mortality in countries that have implemented such a program effectively [19]. This together with the development of better, cheaper, and easier to use diagnostic procedures, has greatly helped in the fight against malaria worldwide.

In the case of veterinary diseases such as Coccidiosis or Neosporidiosis, good litter management in the case of *Eimeria* and better separation of cattle from infected dogs or dog feces for *Neospora*, has reduced the burden of these diseases on farms. In the case of raising chickens, the quality of the shed in which they are grown, the use of good ventilation systems, etc., have all helped to keep the litter dry and thereby reduce the effect of coccidiosis on flock performance. Cattle also need to be raised in well maintained sheds, and constantly monitored for exposure to a variety of pathogens and other environmental factors that can cause disease and stress.

Finally, good nutrition, breeding, and hygiene all play key roles in controlling infection by apicomplexan parasites. In Africa where poor nutrition coupled with multiple infections by immunosuppressive viruses, bacteria, helminthes and fungi, act to greatly exacerbate the rate of malaria transmission as well as the virulence of the infection. In animals, raw materials used in feed may contain endotoxins and other immunosuppressive factors. In addition, animal feed that is produced without proper quality control procedures in place may have lowered levels of key nutrients such as trace metals, amino acids, vitamins, etc. This may have a negative effect on the host immune response, which in turn can greatly effect the performance of flocks or herds.

6. Can we learn to live in harmony with apicomplexan parasites?

What is the explanation behind the fact that some individual animals or humans can harbour parasites even in large number without any detremental effect, while in others there are severe pathological consequences even from a light infection? One possible explanation is immunotolerance. For example, if cerebral malaria caused by *Plasmodium falciparum* in humans is at least in part due to an overly active immune system, than the induction of immunotolerance may reverse this pathogenic effect in the brain. The question is how to induce immunotolerance in a manner that benefits the host, without causing an increase in parasite load and as a result heightened pathogenicity?

In this regard, Ecoimmunology can be applied to ask the question what is the ideal type, quantity and quality of the immune response in controlling parasitic diseases, and what is an acceptable parasite load. From this point of view it can be argued that the complete killing off of all parasites is not necessarily good for the host; indeed host fitness may be maximized at a point of low parasite load together with only a medium or even relatively low immune response [20].

As mentioned above, the immune system can act as a two edge sword being both protective and pathological. Similarly, hosts that are exposed to parasites in a controlled manner can benefit from the induction of the appropriate immune response. The question from an ecologocal perspective is what is the nature of the host-parasite relationship and what drives it towards either harmony or one of disharmony and disease.

A good example of this is the application of the vaccine against *Eimeria*, CoxAbic®. This vaccine was developed in order to control the rate of parasite transmission in a chicken house and not to try and eradicate the parasite. Results from field studies carried out around the world showed that chicks from vaccinated hens, produced fewer numbers of oocysts during the first 3 weeks of growth due to the maternal antibody, and after the antibody waned they maintained a low level of oocysts in the litter that rather than causing disease in the birds induced an appropriate, protective immune response [8]. Thus, by inhibiting parasite transmission, oocyst numbers decreased by 60-80% in the litter which translated into good health and performance of the flocks.

The other important point to be made is that we must look at these parasitic diseases in the context of the whole population. As seen for CoxAbic®, by reducing parasite numbers in the litter, you can provide disease control for the whole flock. It is still possible that within a vaccinated flock there will be a few individual birds that develop mild disease, but overall there is a marked improval in growth, well-being and performance. Similarly, if we were able to do the same thing for malaria, we would greatly reduce the morbidity and mortality caused by the disease and then when necessary focus on the individuals that still require medical attention and care.

Finally, any measure taken to help control apicomplexan parasites must take into account the possibility that a reduction in parasite burden may induce an increase in parasite growth rates and even parasite virulence. Thus, any new measures must be applied with extreme caution with properly controlled field or clinical trials performed over lengthy periods of time. These parasites have already demonstrated their capabilities in adapting to adverse host and environmental conditions and do everything they can to continue to maintain their presence in human or animal populations. Therefore, well designed mathematical modelling and epidemiological studies must be used to accompany any new parasite control program.

7. Conclusions

Parasites have evolved over millions of years in order to be able to propagate and survive in hostile environments. Inherent in this survival is maintaining a proper balance between growth and development within its host animal or human, with its rate of transmission to new hosts. This also includes the need for the parasite to ensure that it does not cause undo harm to its host so that its own survival is ensured.

With that in mind, the host immune system has also developed in order to fight external pathogens while minimizing the harm caused by an overreaction to antigenic stimuli leading to immunopathology. Thus, simply by increasing the response to antigens which normally elicit an immune reaction is not going to be very effective in controlling infections and may indeed have the reverse effect. New approaches to immunotherapy need to be developed including the induction of immunotolerance to ensure a healthy balance between host and pathogen.

Clearly, environmental and management practices that can help reduce parasite transmission without effecting the virulence of the parasite is a useful practice. However, it must be remembered that by reducing parasite numbers in the environment could induce an increased rate of replication and transmission of these pathogenic organisms. This in turn may lead to a situation in which fewer individuals are infected, however, the pathogenicity in that individual may be increased. Therefore, any new practice should be implemented with caution and continuous monitoring backed up by well designed epidemiological studies.

Finally, the development of new vaccines and drugs to control apicomplexan parasites has been the goal of researchers for the past 40 years. Large government grants together with support from major pharmaceutical companies, have pushed this field forward greatly, however, we are still a long way off from being able to control malaria and other human and veterinary parasites. Clinical studies for malaria are in progress using existing antigens from both the sporozoite and merozoite stages of development, however, so far the results are not very encouraging. We must learn from this work how to bridge the work in the lab with what happens in the field and use it to develop better means of vaccination and control. In that way, once we do find the achilles heel of the parasite we will know how to implement its use to start to finally win the battle against apicomplexan parasites.

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INTRODUCTION

The present edition is the third number of a Microbiology book series, which belongs to a more general line of books published by Formatex (Badajoz, Spain), aimed at communicating current scientific and technological research in a generalistic-didactic way. In this number, it aims at stimulating the presentation, exchange and dissemination of information and experiences on anti-microbe strategies (against bacteria, fungi or protozoans), in biotic or abiotic environments, in planktonic or adhered states, in biologically specific or unspecific ways, in vitro or in vivo, in a general context marked by the threat posed by the increasing antimicrobial resistance of pathogenic microorganisms. "Anti" is here taken in a wide sense as "against cell cycle, adhesion, or communication", when harmful for human health, industry or economy (infectious diseases, chemotherapy, food, biomedicine, agriculture, livestock, biotechnology, water systems...). It will include topics on antimicrobial resistance, (early) microbial and resistance detection, enhancement of innate defences against pathogens, as well as methods & techniques.

The topics covered are:

Antimicrobial chemistry (experimental and computational). Analytical detection of antibiotics in complex samples.
Synthesis and screening of novel chemical compounds for antimicrobial action. Natural, synthetic and semisynthetic antibiotics. Analogs. Structural determination. *In-silico/ab-initio/de-novo* antimicrobials discovery. New targets for antimicrobials. Rational design of antimicrobials. Bioinformatics and comparative genomics for the identification of antimicrobial targets…

Antimicrobial natural products.
Antimicrobial substances from terrestrial and marine organisms. Antimicrobial peptides. Antimicrobial enzymes. Essential oils. Bioactive phytochemicals. Plant/Herbal extracts. Purification. Structural determination…

Antimicrobials mechanisms of action.
Methods and Techniques.

Antimicrobial resistance. Superbugs. Multi-resistant strains. Emerging and re-emerging pathogens.
Microbial resistance to antibiotics and biocides. Molecular mechanisms. Resistance genes. Prevention of resistance. Surveillance & statistics. Genetics and Proteomics. Emerging and re-emerging bacteria and fungi in humans, animals, and plants. Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate/Resistant *Staphylococcus aureus* (VISA/VRSA), *Clostridium difficile*, *Mycobacterium tuberculosis*, Vancomycin-resistant *enterococcus* (VRE), *Cryptosporidium*, *Plasmodium parasite*, *Plasmodium falciparum*, *Leishmania* species, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Cryptococcus*, *Escherichia coli* O157:H7, *Helicobacter* spp., *Enterobacter sakazakii*, *Serratia* spp., Fluoroquinolone-Resistant *Pseudomonas aeruginosa* (FQRP)...

Antimicrobial microbes.
Microbial-derived toxins. Bacteriocins (colicins, microcins, lantibiotics…). Archaeocins. Biocontrol approach to microbial invasions (probiotics, lactic acid bacteria…). Biosynthesis of antibiotics. Genetic and metabolic engineering. Gene regulation…

Antimicrobial viruses.
Bacteriophages. Phage therapy and biocontrol in humans, animals (agriculture-farm animals, aquaculture), plants, food industry… Materials functionalization with bacteriophages. Using bacteriophages for microbiological detection…

Antimicrobial materials science and surface chemistry. Biofilms.
Antimicrobial, anti-adhesive surfaces & coatings. Microbial adhesion to surfaces. Biofouling. Biofilm formation, control and eradication. Novel characterization techniques. Physical and chemical (inorganic (e.g.silver, copper compounds) and organic) surface modification. Cationic surfaces. Functionalization strategies for polymers, metals, metal oxides, ceramics. Drug-eluting concepts. Biofilms susceptibility to antimicrobials. Antibiotic resistance of microorganisms in biofilms. Genomics and Proteomics...

Antimicrobials in consumer products.
Textiles (hygienic clothing, activewear, medical textiles…), paper industry, active packaging (food industry…), public buildings (hospitals, schools, restaurants, day care centers, nursing homes…). Safety and toxicological aspects…

Antimicrobial physics.
Exploitation of physical properties for killing/inactivating microbes: surface tension (nano emulsions), radiation, ultrasounds, temperature, specific properties of nano-materials (nano-particles, nano-tubes/wires, nano-crystals, nano-grained materials…). Resistance to physical agents…

Non-antibiotic biocides. Hygiene and Sterilizing.
Disinfectants, antiseptics, preservatives… Mechanism of action. Resistance to non-antibiotic biocides. Combination of physical and chemical treatments. Hygiene and Sterilizing. Sanitizers. Regulatory issues. Good practices…

Techniques and Methods.
Susceptibility Testing. Rapid microbial and resistance detection. Detection of antibiotics in environmental samples. Microscopy, microanalysis & spectroscopy, single-cell studies, high-throughout studies, nanomechanical studies, microfluidics, lab-on-a-chip concepts, miniaturized science, analysis of microbial surfaces, heterogeneity, statistics. Interaction of antimicrobial drugs with model membranes. Analytical techniques…

The Intelligent war.
Interfering microbe-microbe communication (quorum sensing) as antimicrobial strategy.

Strengthening of innate immune system as antimicrobial strategy.
Immunotherapy, immunomodulating agents, cytokines (interleukins, colony-stimulating factors, interferons…), hormones… Novel vaccines for preventing or treating disease…

Antimicrobials evaluation. Pre-clinical and clinical trials.

Public awareness, learning & teaching, influence on policy-makers. Regional regulatory frameworks and experiences on antimicrobials.

We hope that you will find the articles included in this third edition interesting and stimulating, and look forward to receiving new proposals for future editions,

A. Méndez-Vilas, Editor

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