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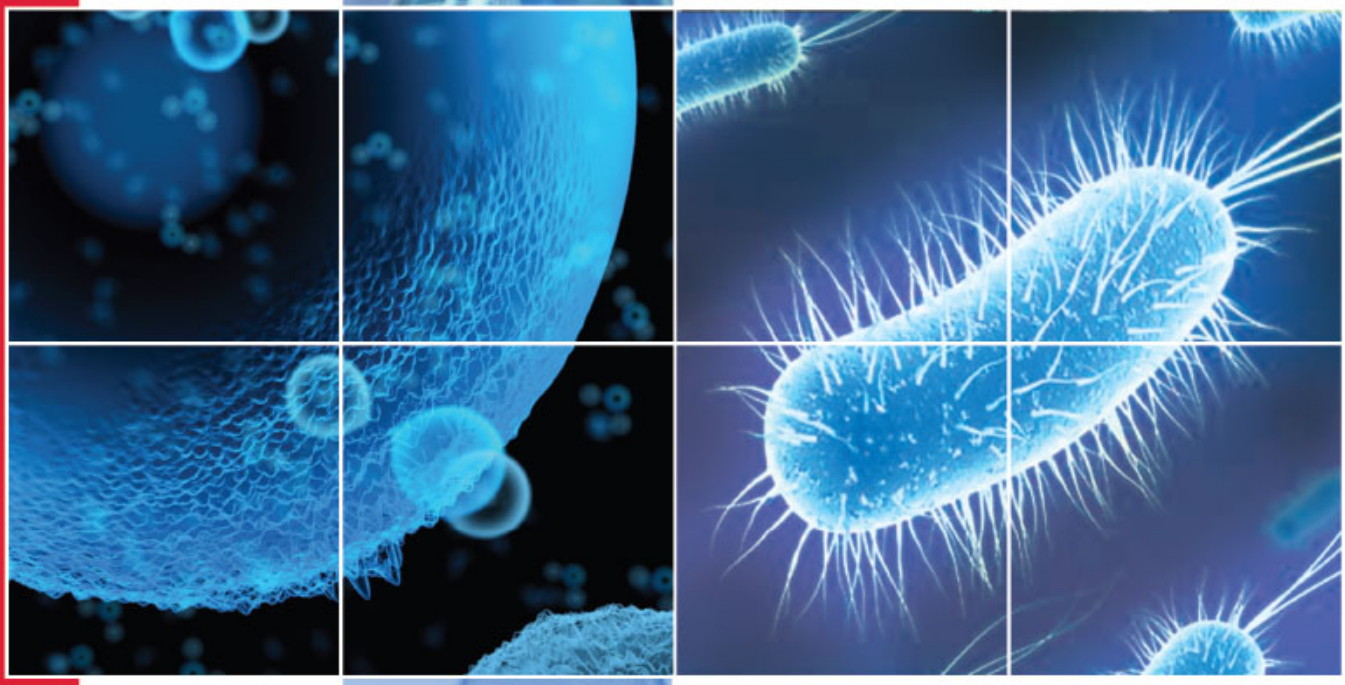
Frontiers in Anti-Infective Drug Discovery

Volume 5

Editors:

Atta-ur-Rahman, *FRS*

M. Iqbal Choudhary



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Frontiers in Anti-Infective Drug Discovery

(Volume 5)

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PREFACE

Infections caused by microorganisms, viruses, and parasites are among the most important challenges faced by the human race. The UN global conference on anti-microbial resistance in 2015 highlighted the need of a global response to tackle epidemics, and emerging drug resistance. The Ebola outbreak of 2013 in West Africa resulting in a heavy death toll, exposed the weaknesses in the current global healthcare systems. Unfortunately, despite tremendous human sufferings as well as the enduring threats to human survival due to infections, the efforts of the pharmaceutical sector toward the development of new anti-infectious agents are less than adequate. Global healthcare research on infections is largely financed by the public funds, which are decreasing world over. This situation demands urgent attention of all stakeholders.

The 5th volume of the book series entitled, "*Frontiers in Anti-infective Drug Discovery*", comprises six reviews focussing on three broad fields *i.e.* molecular mechanism of infections and target identification for drug discovery and development, the use of various natural agents and their derivatives against various infections in humans and livestock, and the use of natural antimicrobial agents in food processing. These articles are contributed by leading practitioners in this field.

Yuichi Itto has contributed a comprehensive review of the recent literature on the physics of diffusion of viruses in the cytoplasm of livings cells. The aim was to present a kinetic theory for the infection pathways of viruses in the cytoplasm of cells. The review by Furneri *et al.* is focused on the antimicrobial activities of essential oils of various medicinal and aromatic plants, especially against multi-drug resistance bacteria. Del Aguila *et a.l* have contributed a comprehensive chapter on bioactive proteins and peptides derived from food matrices, or released from microorganisms. This review described the antimicrobial properties of various protein and peptides in polymeric food matrices.

Varela *et a.l* reviewed the recent literature on the studies of various efflux pump protein super-families which play a key role in multi-drug resistance (MDR) in bacteria. MDR bacteria pose a major challenge in the treatment of infectious diseases. Understanding the underlying mechanism of drug resistance is the key to develop new therapies. The next chapter by Cariddi *et a.l* is focused on an important aspect of infectious disease prevention and treatment. This involves the use of plant based products in boosting natural defence against infections in livestock. The extensive use of antibiotics in cattle is associated with the emergence of antibiotic resistance and the release of antibiotic residues in dairy and meat products. The review emphasizes on the importance of reinforcing the natural defence against infections by using medicinal plant extracts as well as pure phytochemicals, thus decreasing the reliance and use of antibiotics. In the last chapter, Chordia and Kumar contributed an excellent review on the applications of bioinformatics, computational biology and computational chemistry in the identification of new drug target(s) in pathogenic microorganisms. These drug targets can be enzymes, receptors, ion channels and nucleic acids.

In brief, the above cited reviews contributed by leading researchers in the field make this volume an interesting and useful reading for research scientists and graduate students. We wish to express our gratitude to all the authors for their excellent and scholarly contributions for the 5th volume of this reputed eBook series. We also greatly appreciate the efforts of the entire team of Bentham Science Publishers for efficient processing and timely management of publication. The skills and efforts of Ms. Fariya Zulfiqar (Assistant Manager Publications), and leadership of Mr. Shehzad Naqvi (Senior Manager Publications) & Mr. Mahmood Alam (Director Publications) are especially praiseworthy. We also hope that like the previous volumes of this internationally recognized book series, the current compilation will also receive a wide readership and appreciation.

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CHAPTER 1

Virus Infection Pathway in Living Cell: Anomalous Diffusion, Exponent Fluctuations, and Time-Scale Separation

Yuichi Itto*

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Abstract: Recent developments about physics of diffusion for the infection pathway of virus in cytoplasm of a living cell are reported. Specifically, the following three issues are discussed based on the experimental fact that the exponent of anomalous diffusion of the virus fluctuates depending on localized areas of the cytoplasm. Firstly, a theoretical framework developed in view of superstatistics offers a generalized fractional kinetics for describing the infection pathway of the virus over the cytoplasm. There, traditional theory of anomalous diffusion is generalized by introducing exponent fluctuations. Then, the framework explicitly takes into account the existence of two largely separated time scales in the infection pathway. Secondly, a statistical distribution of the fluctuations proposed from the experimental data can be derived by the maximum entropy principle. Thirdly, the motion of the virus over the cytoplasm may obey a scaling law. Consequently, a kinetic theory for the infection pathway of the virus in the cytoplasm is established.

Keywords: Anomalous diffusion, Exponent fluctuations, Generalized fractional kinetics, Living cell, Maximum entropy principle, Scaling law, Shannon entropy, Superstatistics, Time-scale separation, Virus infection pathway.

INTRODUCTION

A number of efforts have been devoted to understanding viruses and related phenomena from the viewpoint of physics (see Refs. [1, 2], for example). In particular, the investigation of the virus infection pathway in living cells may be of obvious importance, for example, for drug delivery based on virus-based carriers [3].

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Just a little more than a decade ago, the infection pathway of adeno-associated viruses in living HeLa cells has experimentally been studied by making use of the technique of real-time single-molecule imaging [4 - 6]. Here, the adeno-associated virus is a small virus particle, and the HeLa cell is a line of human epithelial cells. In the experiments, the virus is labeled with fluorescent dye molecule, and the fluorescent virus solution of low concentrations is added to a culture medium of the living cells. According to the experiments, the fluorescent virus is internalized into cytoplasm of the cell with endosome formation. Here, the endosome is a spherical vesicle, and the virus is contained in it. Subsequently, the virus inside the endosome moves through the cytoplasm and is released from the endosome, resulting in transport of the virus into nucleus of the cell (see Fig. **1**).

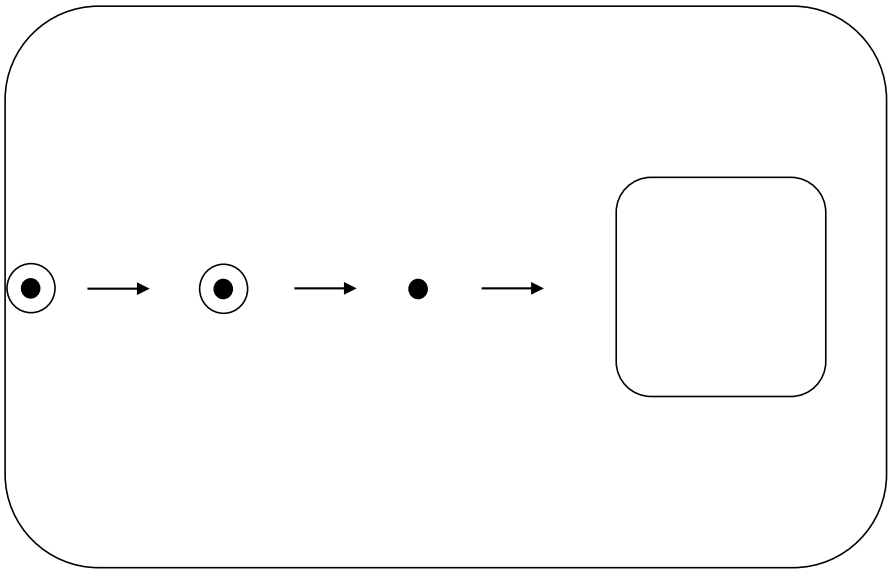

Fig. (1). Schematic description of an overview of the infection pathway of the adeno-associated virus in the cytoplasm of the living HeLa cell. The dot stands for the virus, whereas the circle depicts the endosome. The large and small boxes represent the cell and nucleus, respectively. The arrow indicates a step of the infection pathway.

Consequently, it has been shown that the virus exhibits stochastic motion inside the cytoplasm in two different forms: one is the free form, and the other is the form being contained in the endosome. Quite interestingly, an exotic and certainly remarkable phenomenon has been observed based on analysis of the trajectories of the viruses.

Let $\overline{x^2}$ be the mean square displacement in stochastic motion of a particle, which offers the diffusion property of the particle. In general, the property is

characterized by the relation that $\overline{x^2}$ behaves for large elapsed time, *t,* as

$$\overline{x^2} \sim t^{\alpha} \quad \textbf{(1)}$$

Normal diffusion observed in Brownian motion has the value $\alpha = 1$, otherwise the case with $\alpha \neq 1$ is referred to as anomalous diffusion: subdiffusion (superdiffusion) if $0 < \alpha < 1$ ($\alpha > 1$). This means that the particle in the case of subdiffusion (superdiffusion) diffuses slower (faster) than normal diffusion. Remarkably, the experimental observation mentioned above shows that the trajectories of the viruses exhibit not only normal diffusion but also subdiffusion. However, what is truly remarkable is the following fact [5]: in the case of subdiffusion, the exponent, α, fluctuates between 0.5 and 0.9, depending on localized areas of the cytoplasm. It is noted [5] that this may not be due to the forms of existence of the virus (*i.e.*, the free or endosomal one). Thus, this phenomenon highlights *heterogeneity* of diffusion of the virus. (In a recent work [7], such a phenomenon has been discussed for anomalous diffusion of influenza virus in a living cell. This naturally leads to an interesting question if other viruses exhibit heterogeneous diffusion.) This heterogeneity, in turn, is in marked contrast to traditional anomalous diffusion [8] widely discussed for a variety of physical systems, a short list of which includes particle motion in turbulent flow [9], transport in amorphous solids [10], the flow of contaminated vortex in fluid [11], aqueous solutions of gelatin [12], chaotic dynamics [13], rotating flow [14], porous glasses [15], and gold nanocrystal [16].

Now, in modern statistical mechanics of complex systems, superstatistics [17], which has already been anticipated [18 - 20], has been receiving great attention as a possible theoretical framework for describing nonequilibrium complex systems with different dynamics on two different time scales. Its idea has also been examined in various disciplines, examples of which are tracer particles in turbulence, ecosystems with hydro-climatic fluctuations, highway traffic flows, *etc.* [21 - 27].

The framework of superstatistics is as follows. Consider a Brownian particle moving through a fluid environment with varying inverse temperature, β, on a large spatial scale, which is a prototype system in superstatistics [17, 19]. This system is then divided into many small spatial “cells”, each of which is in local equilibrium state characterized by each value of β. So, a local equilibrium state of the Brownian particle in a given cell is described by the ordinary Boltzmann-Gibbs distribution:

CHAPTER 2

The Antimicrobial Activity of Essential Oils Against Multi-Drug-Resistance Microorganisms: A Review

Pio Maria Furneri[1,*], Virginia Fuochi[1], Edmondo Lissandrello[1], Giulio Petronio Petronio[1,2], Massimo Fresta[3] and **Donatella Paolino[4]**

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Abstract: The use of medicinal plants probably dates back thousands of years. There is archaeological evidence that dates back to their first use, probably to 60,000 years ago. The main products of the plants, which have shown antimicrobial activity, can be classified as phenolics, terpenoids, essential oils (EOs), alkaloids, lectins, polypeptides, and polyacetilenes. Among plant extracts, the essential oils have been used in traditional medicine as therapeutic remedies in the past thanks to their pharmacological properties and their therapeutic importance has been discussed on numerous occasions in the literature. According to the literature, it is known that some EOs possess good antimicrobial activity even against multi-drug resistant (MDR) strains and it has also been seen that some EOs can improve the activity of antibiotics, reducing the dose and toxicity, when used in combination. This review will discuss the antimicrobial activity of EOs with particular attention on their components that can have biological applications, and attention will be focused on those EOs that have shown an activity against MDR microorganisms.

Keywords: Essential oils, Extremely drug resistant (XDR), Multi-drug resistance (MDR), Pan drug resistant (PDR), Susceptibility.

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Atta-ur-Rahman & M. Iqbal Choudhary (Eds.)

INTRODUCTION

The use of medicinal plants probably dates back thousands of years. There is archaeological evidence that dates back to their first use, probably to 60,000 years ago; as evidenced by the discovery of a Neanderthal man skeleton buried with extracts of herbs, among which was yarrow, known as a medicinal herb and from which an essential oil for aromatherapy is extracted [1]. Although the first archaeological evidence of the use of plants as “healing agents” was found in France in the paintings found in Lascaux Cava dated to between 25,000 and 13,000 B.C. [2].

The first written findings on the use of plants for medical purposes dates back to 5000 B.C. and consists of a Sumerian stele of clay found in Nagpur. It describes 12 recipes for preparing drugs from more than 250 plants including poppy, mandrake and henbane [3].

The “history" of the use of medicinal plants continues with the Egyptians about 2,800-2,900 years ago, although the first historical relic is the Ebers Papyrus written over a thousand years later [3, 4]. Moreover, the use of medicinal plants has been known in Traditional Chinese Medicine in the same periods [3, 5]; however, the first manuscript was written more than 2,000 years later [5].

Successively, Hippocrates, around 500 B.C., described about 400 medicinal plants [2, 5 - 6]. In 300 B.C., Teophastros, successor of Aristotle in the Peripatetic school, wrote “Περὶ φυτῶν ἱστορία” (Peri phyton historia) in Latin “*Historia plantarum*”, that is made up of nine volumes and is considered the first scientific botanical book. Particularly, in the ninth book the author described medicinal plants [2, 5 - 6]. Pedanius Dioscorides, who lived in the first century A.C., wrote another important work in five books "*De Materia Medica*" [5, 7]. Theophrastus and Dioscorides, with their works, influenced all scientists that followed, laying the foundations of modern botany and herbal medicine [9].

The main products of the plants, which have shown antimicrobial activity, can be classified as phenolic, terpenoids, essential oils, alkaloids, lectins, polypeptides, and polyacetilenes [7].

Among plant extracts, the essential oils have been used in traditional medicine as therapeutic remedies thanks to their pharmacological properties [8] and their therapeutic importance has been discussed on numerous occasions in the literature [9 - 21].

Essential Oils are represented by mixtures of compounds, mainly volatile ones, resulting from the secondary metabolism of aromatic plants [22]. The International Organization for Standardization (ISO) defines EOs as products obtained from raw vegetable material, either by distillation with water or steam, or from the epicarp of citrus fruits by a mechanical process, or by dry distillation [23].

Most of the molecules present in EOs possess various biological activities: antimicrobial, antiseptic, anti-inflammatory, anti-pain, anticancer and/or tissue regenerative [24 - 26]. Other uses are preservatives, pesticides, flavors in food, drug and cosmetic components [26].

In fact, some natural products contain several bioactive molecules that synergistically provide therapeutic efficacy [27, 28]. For these reasons, these compounds are promising products in several application fields: medical, pharmacological, feed, and cosmetics, and often they are used as alternatives to synthetic and traditional pharmacological preparations.

Antibiotic resistance, despite all the attempts to contain the problem, has been growing over the years [29 - 32]. It has been shown that since the beginning of the 1930s *Staphylococcus aureus* strains resistant to penicillin have been described [33], due to an enzyme called penicillinase [34]. The route of multi-drug resistance (MDR) began in 1959, with the isolation of a *Shigella dysinteriae* strain, resistant to many drugs: in fact, the term MDR was coined in this context. [35]. Over the years, the spread of resistance has led to the creation of new definitions such as “extremely drug resistant” and “pandrug resistant” [36 - 44], and these definitions have prompted the need for a consensus among researchers [45]. A timeline of the main resistance findings since 1940 has been provided by the CDC in the 2013 report [46]. As regards the purpose of this review the most significant dates are 2000 for the appearance of *Mycobacterium* XDR and 2004 for the appearance of *Acinetobacter* PDR [46].

In the US in 2011 of 10,528 TB cases 1,024 were due to strains resistant to first-line drugs [47], of them 124 were MDR and 6 XDR [46]. In his report of 2015, the WHO estimated 190,000 deaths due to *Tuberculosis* MDR in 2014 [47], and it is probable that this number will continue to rise in the next few years.

In the US, each year, about 12,000 cases of nosocomial *Acinetobacter* are registered, of which about 7,300 are due to MDR strains, and among these about 500 are deadly [46].

CHAPTER 3

Natural Antimicrobials in Food Processing: Bacteriocins, Peptides and Chitooligosaccharides

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Abstract: Studies on bioactive proteins and peptides, as well as their potential applications, have continuously increased over the last 20 years. They can be found in all living organisms, from prokaryotes to eukaryotes, and have been detected in different food matrices, maybe the most useful and reliable sources of these molecules. These proteins are referred to as bioactive compounds since they can modify several cellular bioprocesses in order to improve human health human health. Bioactive molecules can occur naturally or can be released from a principal protein after chemical or enzymatic hydrolysis or food fermentation. Bioactive peptides and proteins derived from food matrices or released from microorganisms can present intrinsic antihypertensive, hormone-like, antimicrobial, anti-cancer or antioxidant activities. There is a large demand for natural preservatives and for minimally processed food, researchers have intensified the search for bioactive peptides and proteins, especially those with antimicrobial properties, which are powerful substitutes for conventional food preservatives. This chapter describes the features of antimicrobial peptides and their combination to polymeric materials for food preservation by preventing microorganism proliferation. For this purpose, the bioactive molecules are complexed to chitosan bioactive molecules with chitosan biofilms, creating an antimicrobial packaging. Despite the changes that can occur in the physical properties of these biofilms, the incorporation of antimicrobial peptides to bioplastic biofilms could guarantee the quality and safety of foodstuffs, contributing in extending their shelf life.

Keywords: Antimicrobial mechanism, Antimicrobial spectrum, Bacteriocins, Bioplastic films, Biopolymer, Chemical compounds, Chitosan, Food preservation, Food safety, Natural packing, Peptides.

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INTRODUCTION

It is known that proteins are versatile molecules with many functions in the metabolism. They act in the defense, or immune system, as enzymes, carriers and signaling mediators, among other important metabolism functions [1]. Bioactive peptides within protein sequences are able to affect cell activities resulting in the promotion of health [2]. These peptides can present multiple bioactivities, which includes antimicrobial [3, 4], antioxidant [5, 6], antihypertensive [7] anti-thrombotic and immunomodulatory activities [8, 9].

Some authors consider that the first report on antimicrobial peptides (AMPs) occurred when Fleming [6] identified a substance, lysozyme, in human epithelial cells, mucosae and fluids, which showed the ability to cause bacteria death through lysis. This antimicrobial agent is produced not only by those parts of the body in direct contact with the environment, but also into circulating fluids, such as blood cells, inner organs and hemolymph. It was evidenced in the early 90s that lysozyme also exerts a non-enzymatic mechanism against microorganisms, similar to AMPs [10, 11].

Several AMPs have been identified and isolated from all kinds of living organisms, including animals, plants, bacteria, protists and fungi. They show broad antimicrobial activity against both Gram -positive and gram-negative bacteria [12]. The number of identified antimicrobial peptides has increased over last twenty years, according to the number of publications displayed in Fig. (**1**). Currently, already more than 1500 types of AMPs found in different organisms have been described [13].

Antimicrobial peptides have been proven to be powerful tools for application in medical area when incorporated to medical instruments and in food industry, ensuring the food safety and quality [14].

AMPs can replace antibiotic use and contribute to therapy against bacterial and fungal infections, and they represent a new model for the development of effective drugs against pathogens resistant to conventional antibiotics [15 - 18].

These peptides are effective against several classes of microorganisms as bacteria, virus, protozoa and fungi [19, 20]. They can be readily synthesized in a flexible manner and with low-power and biomass, due to their small size [21]. Many are synthesized or activated by proteolysis from specific proteins [22].

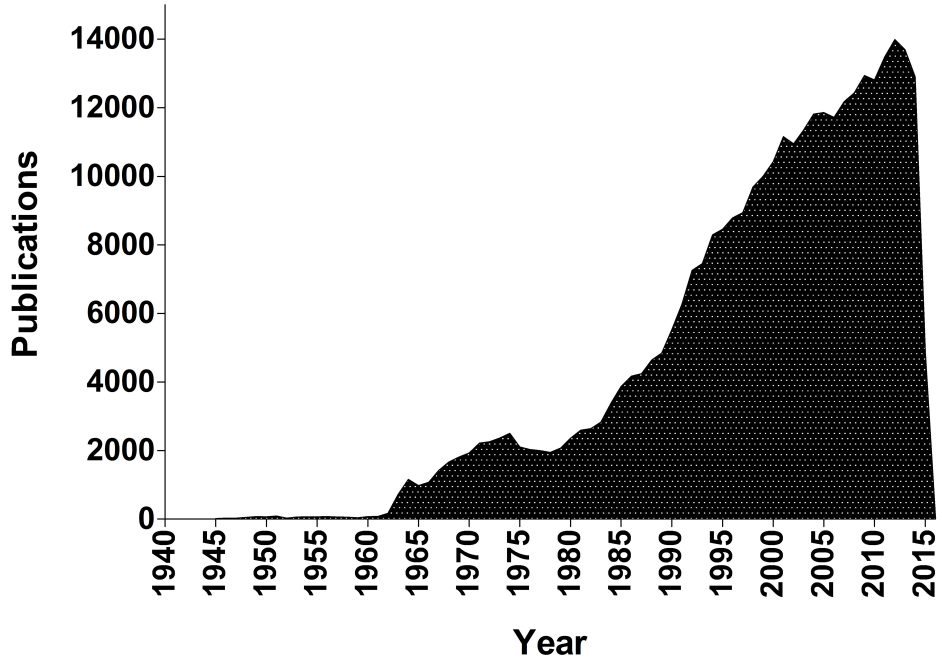

Fig. (1). Number of publications on antimicrobial peptides, from 1940 until nowadays.
The graph is the result of a PubMed search using "antimicrobial peptide" as the keyword. Data was obtained at the sciencedirect.com website – December /2015.

With the increasing number of new AMPs, data is being accumulated in this area and there is the need to organize the information in databases. Existing AMP sequences and/or structures are available in at least 18 active databases (Table **1**), which exhibit AMP entries from diverse origins or restricted to a particular AMP family or source [23].

Table 1. Updated list of the existing database dedicated to antimicrobial peptides.

Database	Website	Type
RAPD	Inactive	Recombinant AMPs
PhytAMP	http://phytamp.pfba-lab-tun.org/main.php	Plant AMPs
BACTIBASE	http://phytamp.pfba-lab-tun.org/main.php	Bacteriocin natural antimicrobial peptides
Defensin Knowledgebase	http://defensins.bii.a-star.edu.sg/	Defensin family
PenBase	inactive	Shrimp penaeidin database

CHAPTER 4

Bacterial Resistance Mechanisms and Inhibitors of Multidrug Efflux Pumps Belonging to the Major Facilitator Superfamily of Solute Transport Systems

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Abstract: Multidrug resistant pathogenic bacteria pose a serious public health concern as their recalcitrant nature enhances treatment failure of infectious diseases. Several molecular mechanisms are responsible for multidrug resistance in bacteria. A major antibacterial resistance mechanism involves active drug efflux, grouped into transporter superfamilies. Of these, the major facilitator superfamily harbors clinically important drug and multidrug efflux pumps and constitutes a large number of transporters that share similarities in protein sequences, three-dimensional protein structures, energy modes, and evolutionary origin. Multidrug efflux pumps of the major facilitator superfamily in bacterial pathogens compromise the efficacy of infectious disease treatments. Thus, inhibition of these antibacterial efflux pumps is critical in order to circumvent drug resistance and potentially restore the clinical utility of infectious disease chemotherapy. This chapter summarizes bacterial resistance systems and multidrug efflux pumps from the major facilitator superfamily and the nature of efflux pump inhibitors.

Keywords: Antimicrobial Efflux Pump, Bacterial Antimicrobial Resistance, Efflux Pump Inhibitor, Major Facilitator Superfamily, Multidrug Resistance, Transporter.

MECHANISMS OF ANTIBACTERIAL RESISTANCE

Bacterial antibiotic resistance systems have emerged as a problem worldwide

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within the last two decades [1]. Pathogenic bacteria develop antimicrobial resistance using an assortment of cellular mechanisms including alteration of drug targets, decrease in drug permeability across bacterial membranes, inactivation of antibiotics, extrusion of antimicrobials by efflux pumps [2, 3] and biofilm production [4].

Target Alteration

Alterations in the target sites of antibiotics often result from spontaneous mutation of a gene [5]. Resistance to antibiotics like rifamycins and quinolones occurs by this cellular mechanism due to mutations in RNA polymerase and DNA gyrase, respectively. The reduced activity of fluoroquinolones occurs due to the alteration in subunits of topoisomerase IV [5]. Target modification can also occur through enzymes. Resistance to antibiotics like the macrolides (erythromycin), lincosamides (clindamycin), and type B streptogramins (quinupristin) occurs by the Erm enzymes that modify the 23S rRNA of the larger subunit of the ribosome [6]. Modification of a drug's target site may result in reduced binding affinities for β-lactam antimicrobial agents. The altered target affinities for β-lactams are often the result of various penicillin-binding proteins (PBPs) which do not effectively bind beta-lactams and, consequently, no longer inhibit cell wall synthesis in bacteria [5]. Other target site modification includes changes in peptidoglycan precursor, thickening of the cell wall and changes in peptidoglycan layer leading to a decrease in the antibacterial activity of vancomycin [5]. Another interesting finding involves the outer-membrane protein, Tsx, for nucleoside uptake, which when altered by insertion mutagenesis, is relatively impermeable to the gyrase inhibitor albicidin, and thus, resistance to this antimicrobial agent is conferred [7, 8].

Reduced Drug Permeability

Reduced drug permeability is the resistance mechanism in which a given antimicrobial agent cannot gain entry into the bacterium where drug targets are intracellularly located [9]. An important resistance mechanism involves the reduction in the intracellular permeability of a drug by the lipopolysaccharide in the bacterial cell wall and porin channels that are located within the bacterial outer membrane [10]. Lipopolysaccharide consisting of lipid A, a core polysaccharide, and O-antigen, is principally responsible for conferring an impermeable property towards hydrophobic antibiotic and detergent molecules [9]. The porin channels allow small molecules, such as antibacterial agents, to enter into the cell. There are two major porin-based reduced drug permeability mechanisms: there is either (a) an alteration in the expression of these outer membrane proteins that results in

failure to integrate into the outer membrane or (b) an alteration in function due to specific mutations [11].

Drug Inactivation

Inactivation of antibiotics occurs by various mechanisms such as the enzymatic degradation of antimicrobial agents, group transfer systems and redox processes [12]. A classic example of enzymatic hydrolysis is the hydrolytic deactivation of the β-lactam ring of the penicillins and cephalosporins using a group of bacterial enzymes called β-lactamases [10]. The macrolide esterases hydrolyze the lactone ring, thus inactivating erythromycin A and oleandomycin [13]. Other antibiotic hydrolyzing enzymes such as epoxidases hydrolyze fosfomycin [14]. The second mechanism involving antibiotic degradation involves a structural alteration of the antibacterial agents through the transfer of chemical functional groups through, for example, acylation of aminoglycosides and chloramphenicols, phosphorylation of macrolides and rifamycin, thiolation of fosfomycin, or ribosylation of rifamycin [15]. A less common mechanism of antibiotic inactivation involves the redox process, which enzymatically uses a flavin-dependent monooxygenase determinant, TetX, that confers resistance to the tetracycline class of antibiotics [16].

Antimicrobial Efflux Pumps

Antimicrobial efflux pump systems are composed of integral membrane proteins and are present not only in the biological membranes of Gram-negative and Gram-positive bacteria but also in the plasma membranes of eukaryotic cells [17]. The genes that encode selective antimicrobial agent efflux pump proteins may be located on extrachromosomal elements like plasmids or transferable genetic elements, while those determinants that encode multidrug efflux pumps are generally located on the chromosome of bacterial cells [18]. Bacterial efflux pumps recognize harmful agents that have reached the periplasm or cytoplasm after penetrating the cell wall of the organism and extrude the drugs before they are able to reach their intracellular targets [19]. Bacterial drug efflux pumps can be either specific, exporting only one antimicrobial agent or a class of antimicrobials, or non-specific, exporting different classes of antibiotics [20, 21]. The major superfamilies of antimicrobial efflux proteins [22] (Fig. **1**), will be discussed in more detail later.

CHAPTER 5

Medicinal Plants as Immune Response Enhancers to Prevent Infectious Diseases of Veterinary Interest

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Abstract: Mastitis is considered worldwide as the disease of cattle that causes severe economic losses in dairy industry worldwide and is usually associated with the presence of infectious agents as bacteria. Bacterial pathogens have been classified in contagious, environmental and opportunistic pathogens. Antibiotic therapy is one of the routine treatments for mastitis. However, antibiotics are moderately effective and their indiscriminate use leads to resistant strains. In addition, residues remain in milk with implications for human health.

Therefore, one of the objectives of the dairy industry is to reduce the use of antibiotics in animals food producing. The mammary gland has defense mechanisms against invading pathogens.

The incidence of mastitis increases when these mechanisms are impaired. Polymorphonuclear neutrophils (PMN), macrophages and lymphocytes play a very important role in the defense against mastitis. These cells regulate both the innate and adaptive response. Alternative therapies are conducted in order to both reinforce the antimicrobial therapy and to increase the natural defenses of the mammary gland. The application of immunomodulatory compounds to stimulate the immune response of the mammary gland is one of the most innovative alternative strategies studied today.

In this context, immunomodulators compounds derived from medicinal plants appear as an effective alternative therapy. Several studies have reported that ginseng saponins or ginsenosides of *Panax ginseng*, extracts of *Tinospora cordifolia* or *Taraxacum mongolicum*, flavonoids of *Rosa agrestis* among others, have stimulatory effects on immune response of the mammary gland with potential use in the treatment of bovine

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mastitis. Strategies to enhance the immune response of the udder will heavily impact the animals' ability to resist pathogen infection.

Keywords: Active Metabolites, Alternative Therapy, Bovine Mastitis, Cattle, Control of Infection Diseases, Immunomodulators, Intramammary Infections, Immune response, Microbial Agents, Medicinal Plants.

INTRODUCTION

Bovine Mastitis

Bovine mastitis is one of the infectious diseases of the dairy farm that causes significant economic losses in the dairy industry all over the world, causing decreased milk production and low-quality milk [1, 2]. The losses are ascribed to a decrease in the milk production, the costs of the veterinarian treatments and the removal of the infected cows [2 - 4].

Agents causing mastitis are frequently characterized as either contagious or environmental, depending on their primary reservoir and way of acquisition. Mastitis can be classified as acute or chronic; clinical or subclinical according to its duration. Clinical mastitis is based upon the severity of the inflammatory response characterized by visible abnormalities that result in either swelling or heating of the udder or milk production with an abnormal appearance with the presence of flakes or clots. Subclinical mastitis is inflammation with no changes in the udder or the milk. Generally, cows with subclinical mastitis produce fewer liters of lower quality milk [5 - 7].

Mastitis Pathogens

Mastitis is usually associated with the presence of infectious agents. A large number of microbial agents come into contact with the udder and have the opportunity to enter into the mammary gland *via* the teat canal [8].

There are more than 80 causative agents of mastitis; including species of bacteria, fungi, mycoplasma and algae [9]. However, most infections are caused by bacteria. Bacterial pathogens are common and have been classified in a) contagious pathogens b) environmental pathogens and c) opportunistic pathogens according to the way of transmission [10].

Contagious agents are frequently taken from teat skin. They are transmitted from infected to uninfected quarters and from animal to animal mainly during milking *Staphylococcus aureus* is the contagious species most frequently isolated. The

environmental pathogens have the reservoir in the environment around the animals [11].

These organisms are a heterogeneous group and include genera as *Enterococcus* and *Streptococcus* and coliform bacteria [12].

Environmental pathogens can cause mastitis infection when the cow′s defenses are depressed or hygiene standards are not properly practiced during and after milking [13].

Other group consists of coagulase-negative *Staphylococcus* (CNS), which are considered opportunistic pathogens because although they are members of the normal flora of the udder and teat skin, they can also cause infections of the teat canal and the mammary gland. The distribution of different species of CNS within dairy herds reflects in part management practices [14].

There are yeasts and even bacteria that cause mastitis less frequently. Infection occurs when environmental conditions change and increase exposure to these organisms. *Arcanobacterium pyogenes*, *Nocardia asteroides*, *Bacillus cereus*, *Pseudomonas aeruginosa*, anaerobic bacteria species, fungi and yeasts are some examples [15].

Control of Intramammary Infections

Current programs used in the control of bovine mastitis are based on milking hygiene, including disinfection after milking, lactating nipples antibiotic therapy, the beginning of the dry period and disposal of chronically infected cows. Although antibiotics have a positive impact on dairy systems benefiting udder health and milk production, their indiscriminate use leads to the emergence of resistant strains, leaving residues in milk with implications for human health. Likewise, the application of these measures has led to considerable progress in the control of infectious pathogens. However, several studies have shown that when it was possible to reduce the prevalence of contagious pathogens, the proportion of intramammary infections by environmental pathogens increased [16]. As these strategies for prevention and/or treatment are not 100% effective to combat mastitis and thus ensure milk safety, the research has been directed towards the development of vaccines. However, attempts worldwide to produce a vaccine have not been successful to completely prevent the occurrence of new infections due not only to diversity of contagious and environmental pathogens that colonize the epithelium of the mammary gland, but also to the difficulty to generate an appropriate and effective immune response [17, 18].

CHAPTER 6

In Silico Approaches for Determination of Drug Targets

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Abstract: It is being realized that most of the pathogens responsible for causing diseases in human and other animals have become resistant to general antibiotics. Therefore, there is more emphasis on the development of specific drugs in present day researches. Bioinformatics has played an important role in this field and due to which cost of drug development is curtailed even upto 60 to 70%. During work on specific drug development, important part is determination of drug target(s). A drug target is a biological molecule whose activity is altered by drug that results in desirable therapeutic effect. Drug targets are mainly enzymes, receptors, ion channels or nucleic acids. Identification of drug targets is very complex process during early drug discovery. After genome sequencing, bioinformatics design essential tools are used for *in silico* drug target identification. These include tools for genome/ proteome analysis, similarity searching, EST identification, structure prediction, functional prediction, localization prediction, pattern matching, pathway mapping, network analysis, protein-protein interaction and many more. Using combination of these tools, different approaches are designed to find the drug targets. In this chapter, we have tried to describe some of these approaches with the tools that are used for identification of drug targets. In addition, we also discussed the results obtained in many cases by applying these approaches.

Today, drug discovery is relying on computational methods to accelerate the identification of potential drug targets. This acceleration leads to fast drug discovery process. These computational methods are used based on the available data and resources of the pathogen and disease. However combinations of approaches are also used to fully characterize the drug target. Once a drug target is identified, it is validated by several wet lab techniques.

Keywords: Drug targets, Identification, *In silico*, Network, Protein, Subtractive genomics, Validation.

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INTRODUCTION

Drug design and discovery is a lengthy, intensive and inventive process for finding new medications based on the knowledge of a biological target. It is linear and progressive process that starts with target and lead discovery, followed by lead optimization and pre-clinical studies. The target discovery identifies and validates a suitable target that can be used to treat a disease, whereas lead discovery identifies novel chemical molecules that act on those targets. The development of a new drug requires a technological expertise, human resources and huge capital investment. Traditional method of drug discovery relies on trial-and-error testing of chemical substances on cultured cells or animals and matching the apparent effects to treatments. In traditional method of drug discovery, many protocols of testing and manufacturing standards need to be followed before the drug comes in the market and used by the public. In fact, sometimes it fails during the process to allow its entry into the market. All these factors just increase the cost for a new chemical entity research and development. This traditional method is challenging, expensive, and time consuming. The process is too long starting from target identification and validation, lead identification and validation, and preclinical and clinical studies. The complete process takes nearly 15 years. In contrast, computational drug design process has cut down the time to nearly 3 to 5 years (Fig. **1**). Usage of Bioinformatics tools and software in drug designing process made positive effect on overall process and this can accelerate various steps of drug designing and reduce the cost and over all time [1].

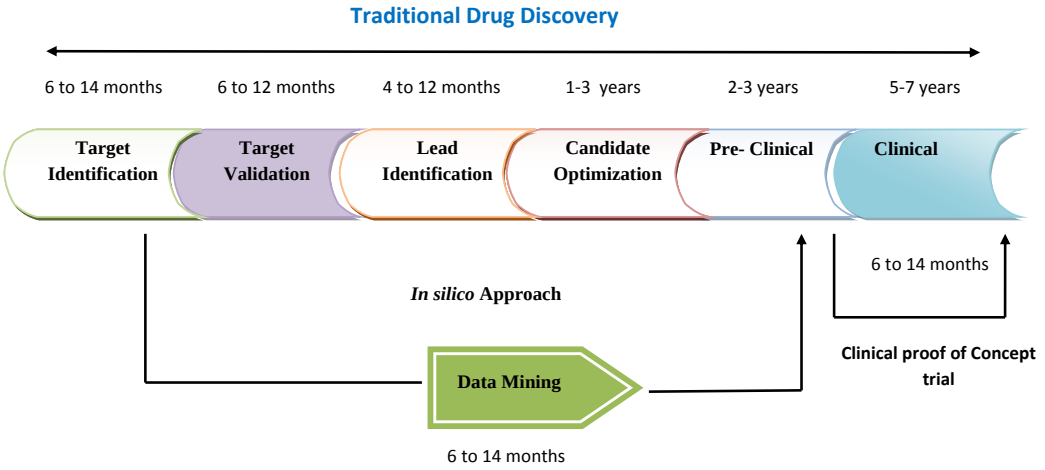

Fig. (1). Comparison of traditional and computational drug discovery process.

The most important part of any successful drug discovery is the identification of drug target. The target identification is the foremost step for biomarker identification and drug discovery process. Previous records showed that improper drug target selection led to the high failure rate of drug development. A good target needs to be efficacious, safe, should meet clinical and commercial requirements and must be 'druggable'.

A 'druggable' target is accessible to the putative drug molecule that can be a small molecule or larger biological and upon binding elicits a biological response which may be measured both *in vitro* and *in vivo*. A target is a broad term which can be applied to a range of biological entities and may include proteins, genes and RNA to biological phenomena such as molecular functions, pathways and phenotypes. If a good target is identified and validated, it will show the high confidence in the relationship between target and disease that will allow to find whether target modulation will lead to mechanism-based side effects. There are many bioinformatics approaches that not only help in identifying targets but also in selecting and prioritizing potential disease targets [2]. Bioinformatics approaches combine biological concepts with computer tools or statistical methods to find the drug target [3].

Here in this chapter, we have described various *in silico* methods for the identification of drug targets and the tools used in these methods. In addition, we have also discussed the results obtained in many cases by applying these approaches. A few drug target databases are also discussed.

PREFERRED PROPERTIES OF DRUG TARGETS

There are certain properties that are preferred for the drug target. In general, a drug target is defined as a macromolecule, which is most often a protein, and whose manipulation could lead to removal of causes or relieving the symptoms caused by the underlying patho-physiology. Additional drug target properties that are preferred include [4]:

i. Essentiality: Drug target should be essential for the pathogen.
ii. Druggability - Its function can be manipulated by an appropriate small molecule.
iii. Process specificity - It should be specific to the disease process or state.
iv. Pathogen specificity- It should be specific for pathogen species/family.
v. Biological Tractability- Target is available in suitable quantity *in vivo*.
vi. Low Mutability - Low mutability leads to lower chances of drug resistance.
vii. Assayability - Suitable methods are available to test the function of the

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