

# Frontiers in Anti-Infective Drug Discovery



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Atta-ur-Rahman, *FRS*

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# **Frontiers in Anti-Infective Drug Discovery**

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## PREFACE

The recent COVID-19 pandemic has further highlighted our human incapacity to control infections. Pathogens of all forms and types are fast learners, and their mutations, spread and virulence can overwhelm the entire health care system within weeks. The COVID-19 pandemic has also exposed our inability to quickly come up with treatment and prevention regimes, despite the tremendous progress in pharmaceutical and biomedical sciences. In the post COVID-19 world major attention to the surveillance, prevention, and treatment of infections of all kinds is expected. Research on infections and anti-infectious drug discovery is already truly interdisciplinary in nature, and is published in journals of diverse disciplines, such as microbiology, molecular and structural biology, genomics, immunology, epidemiology, *etc.* It is imperative that the most exciting discoveries in this field are compiled as critically written reviews in frontier areas.

The aim of the book series “*Frontiers in Anti-infectious Drug Discovery*” is to focus on recent important developments. Experts in various important aspects of anti-infectious drug discovery have therefore contributed review articles on the most recent advancements. Volume 8, like the previous volumes, of this well received book series, comprises eight (8) scholarly written review articles on certain key aspects. These include genomic based identification of new drug targets and metagenomics for antimicrobials; fragment-based approach for drug designing, and of various types of antimicrobials ranging from synthetic analogs against coronaviruses, to bacterial phages against infections, nanoparticle based agents, as well as aptamers.

The chapter contributed by Gisbert and McNicholl focuses on the key advantages of concomitant non-bismuth quadruple therapy for a range of infections caused by *Helicobacter pylori*. Silva-Junior *et al* have presented an interesting review on the discovery and development of bioactive drug leads against the recent pandemic caused by SARS-CoV-2, based on analogs developed during the past SARS and MERS epidemics. Advances and challenges in fragment-based designing of new antibiotics is the key focus of the article by Kwan *et al*, supported by numerous examples. Foodborne bacterial infections are widespread. Ilyina *et al* review the recent applications of phage therapies as alternatives to antimicrobials for the treatment of food borne bacterial infections. Amjad *et al* have contributed a chapter on the applications of subtractive genomics to identify essential genes involved in crucial metabolic pathways of pathogens, and validating their protein products as novel drug targets. Metagenomics has emerged as a key technique for the discovery of novel antibiotics from yet uncultured microbes. The tremendous pool of new antimicrobials in unexplored microbial flora is the focus of the review by Chopra *et al*. Zameer has contributed a chapter on the use of nanoparticles as drug carriers of synthetic and natural antimicrobial agents. In the last chapter, Syed *et al* have touched upon an important new field of the use of aptamers (oligonucleotides or peptide molecules) as novel diagnostic and anti-infective agents.

We would to express our sincere thanks eminent to all the authors for their excellent contributions in this vibrant, and exciting field of biomedical and pharmaceutical research. The efforts of Ms. Fariya Zulfiqar (Manager Publications) and the excellent management of Mr. Mahmood Alam (Director Publications) are also gratefully acknowledged.

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# Eradication of *Helicobacter pylori* Infection with Non-Bismuth Quadruple Concomitant Therapy

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**Abstract:** Background: The main recommended regimens to eradicate *Helicobacter pylori* infection fail in  $\geq 20\%$  of the cases. Several substitutes for triple therapies have been proposed, and non-bismuth quadruple therapy is one of the most widely used.

**Aim:** To systematically review the efficacy of non-bismuth quadruple regimen (proton pump inhibitor, clarithromycin, amoxicillin and a nitroimidazole) in the eradication of *H. pylori* infection.

**Methods:** Bibliographical searches were performed in MEDLINE/EMBASE and relevant congresses. We pooled studies evaluating the concomitant regimen, and of the randomized controlled trials comparing concomitant vs. standard triple therapy, and concomitant vs. sequential therapy.

**Results:** Fifty-five studies were included (6,906 patients). The meta-analysis showed that concomitant regimen offers an overall eradication rate of 87%. A sub-analysis of studies comparing one-to-one concomitant and triple therapies showed an *odds ratio* of 2.14 (95% CI=1.51-3.04) towards higher efficacy with concomitant regimen. This figure increased up to 2.41 (95% CI=1.80-3.24; 85% vs. 72%) when comparing arms lasting the same number of days. We also sub-analyzed the comparative efficacy between non-bismuth quadruple concomitant and sequential treatments, and concomitant achieved an *odds ratio* of 1.49 (95% CI=1.21-1.85) towards higher eradication results than sequential regimen.

**Conclusions:** Non-bismuth quadruple (concomitant) therapy achieves high efficacy in *H. pylori* eradication, superior to standard triple and sequential therapy. Concomitant may be more appropriate than sequential therapy for patients with clarithromycin and/or metronidazole resistance. Higher acid suppression and/or longer duration are optimizations that can increase even more its efficacy.

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**Keywords:** Amoxicillin, Clarithromycin, Concomitant therapy, *Helicobacter pylori*, Metronidazole, Non-bismuth quadruple, Proton pump inhibitor, Resistance, Sequential therapy, Treatment.

## INTRODUCTION

Approximately fifty percent of the world population is infected by *Helicobacter pylori*, a bacterium linked to a broad range of upper gastrointestinal conditions such as gastritis, peptic ulcer disease, and gastric cancer [1]. The most commonly used therapy for the eradication of *H. pylori*, traditionally recommended by international consensus, is the proton pump inhibitor (PPI)-based, standard triple therapy, adding two antibiotics (clarithromycin plus amoxicillin or metronidazole) to a PPI [2 - 6]. However, the eradication rates with this regimen have fallen considerably [7, 8]. Previous meta-analyses (with more than 53,000 included patients) showed an efficacy below 80% [9, 10]. Therefore, recent debate has been raised regarding how ethical it is to continue using standard triple therapy, and alternative approaches have been recommended [11]. Although, efforts to improve eradication prolonging triple therapy's duration have been tested, data have not consistently provided significant benefits [12, 13]. Consequently, new combinations to improve treatment of naïve patients remain as an urgent need.

Sequential treatment involving a dual regimen with a PPI plus amoxicillin for the first 5 days followed by a triple regimen including a PPI, clarithromycin, and a nitroimidazole for the following 5 days, was proposed as an alternative [14]. Several randomized clinical trials and meta-analyses have shown that the sequential regimen was more effective than the standard triple [15 - 19]. Therefore, some consensus conferences suggested sequential regimen as a substitute to standard triple for the first-line eradication of *H. pylori* [20]. Nevertheless, results obtained by a meta-analysis by the Cochrane Collaboration [21] concluded that sequential regimen outcomes were heterogeneous, and that many of the latest manuscripts were unable to show any benefit from sequential over standard triple therapy. The conclusions of the meta-analysis were clear even though the pooled eradication rate was 85%, and a potential trend towards reduced efficacy was observed in the last years [21].

Sequential treatment faced another relevant issue, whether sequential administration was really necessary or if the 4 drugs could be given concurrently [14, 22, 23]. Questions were raised regarding the risk of failure to comply with the treatment due to regimen complexity [11, 24] Moreover, the combination of amoxicillin, clarithromycin and a nitroimidazole with a PPI has previously been evaluated as a concomitant regimen in 1998: two research teams, one in Japan and the other in Germany, recommended that this drug combination should be

prescribed as a concomitant 4-drug, 3-antibiotic, known as non-bismuth quadruple therapy [25, 26], providing high efficacy even in short durations (>90% by intention-to-treat in 5-day regimens).

This “non-bismuth quadruple concomitant” regimen has regained presence in recent years [27]. It is easy to convert the standard triple therapy (PPI-clarithromycin-amoxicillin) to concomitant therapy by adding of 500 mg of metronidazole (or tinidazole) twice daily [28]. Beware that “concomitant” (taking all drugs all together) may cause confusion; this term is actually a misnomer, as all *H. pylori* treatments, except sequential therapy, could be called concomitant therapies. Nonetheless, this will be the name used hereafter as it has been the most common denomination in the literature.

## OBJECTIVE

The aim of the present chapter is to perform a critical review of published evidence on the efficacy and safety of concomitant therapy in the eradication of *H. pylori* infection. We will review the following aspects: 1) Efficacy of the concomitant regimen; 2) Comparison between the concomitant regimen and standard triple therapy; 3) Comparison between the concomitant and the sequential therapies; 4) Effects of different variables on the efficacy of concomitant therapy; 5) How could we increase the efficacy of the concomitant treatment? and finally; 6) What are the results with the concomitant treatment in clinical practice? (the experience of the European registry on *H. pylori* management).

## BIBLIOGRAPHICAL SEARCHES

Bibliographical searches were performed in MEDLINE and ENDBASE using the following keywords (all fields): ((concomitant OR quadruple OR concurrent OR ((amoxicillin OR amoxycillin) AND (metronidazole OR tinidazole OR nitroimidazole) AND clarithromycin) AND (“*Helicobacter pylori*” OR “*H. pylori*”). No language restriction was applied. Bibliography from selected manuscripts and reviews were hand-searched to identify further relevant studies. Authors conducted a hand-search of communications from the *American Digestive Disease Week*, the *International Workshop of the European Helicobacter Study Group*, and the *United European Gastroenterology Week*. Summaries of the manuscripts selected in the different searches were reviewed, and screened for exclusion and inclusion criteria. In cases of duplicate reporting of studies or evidently based on overlapping study population, the latest valid report was considered.

## Drug Discovery Strategies Against Emerging Coronaviruses: A Global Threat

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**Abstract:** After the discovery of the infectious bronchitis virus (IBV) in 1932, *Coronaviridae* emerged as a family of viruses constituted of a positive-sense single-stranded RNA ((+)ssRNA) genome. Recently, the Coronavirus disease-2019 (COVID-19), which is caused by a new virus called SARS-CoV-2 (provisionally titled 2019-nCoV), was declared pandemic since it reached global levels of infection. In comparison, this disease spread globally more quickly than previously reported SARS- and MERS-CoV outbreaks. The impacts on global health systems (as well as the world economy, estimated to cost US\$ 1 trillion) highlighted the urgent need to search for efficient pharmacotherapy targeting potential macromolecules from SARS-CoV-2 since there are no licensed vaccines or approved drugs until today. In this chapter, we will demonstrate all strategies that have been used to discover and design bioactive molecules against this viral infection, compiling from classical to computer-aided drug design, including also the drug repurposing. This last, it is based on analogs produced for past outbreaks related to SARS- and MERS-CoV. Finally, we aim to provide valuable information that could be applied for designing new safe, low cost, and selective lead-compounds against these emerging viruses.

**Keywords:** Coronaviruses, Drug Design, MERS-CoV, SARS-CoV, SARS-CoV-2, HCoV.

### INTRODUCTION

*Coronaviridae* term refers to the viruses family known as Coronavirus (CoV), which is potentially contagious to humans and causes severe infection in the res-

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piratory tract [1, 2]. Previously, CoV was responsible for the MERS-CoV outbreak in the Arabian Peninsula, with 2,123 cases and 740 deaths, leading to a high fatality rate of 35%. Furthermore, between the 2002-2003 SARS-CoV outbreaks in Guangdong province (China) infected 8,500 people, causing 800 deaths [3, 4].

On March 11<sup>th</sup>, 2020, WHO declared the so-called new Coronavirus (SARS-CoV-2) as a pandemic virus [5], in which it was first reported on December 8<sup>th</sup>, 2019, in Wuhan, Hubei, China. SARS-CoV-2 is an emerging and severe respiratory infection that causes severe pneumonia. Nowadays, more than 1,8 million individuals have been affected by 215 territories, leading to more than 116,000 deaths. Among these, 3,341 deaths were reported only in China [6 - 9].

The economic impact has reached global and catastrophic proportions, considering that China's production represents about 17% of the world. By comparison, in the past SARS 2003 outbreak, Chinese production represented 4% of the world [10]. Besides, the fact that China is the largest manufacturer and importer of crude oil, has led economists to reduce the annual expectation of global growth [11, 12].

Recently, several works have compiled targets and drugs with activity against Human-Coronaviruses (HCoV) to provide promising alternative treatments, such as potential vaccines, peptides, monoclonal antibodies, and small-molecules [13 - 15]. Also, the publication of the first crystal structure of the main protease (3CL<sup>pro</sup>, also named as 3C or M<sup>pro</sup>) from SARS-CoV-2 obtained by X-ray crystallography (PDB ID: 6LU7, ref [16].) will potentially contribute for developing selective inhibitors against this viral target.

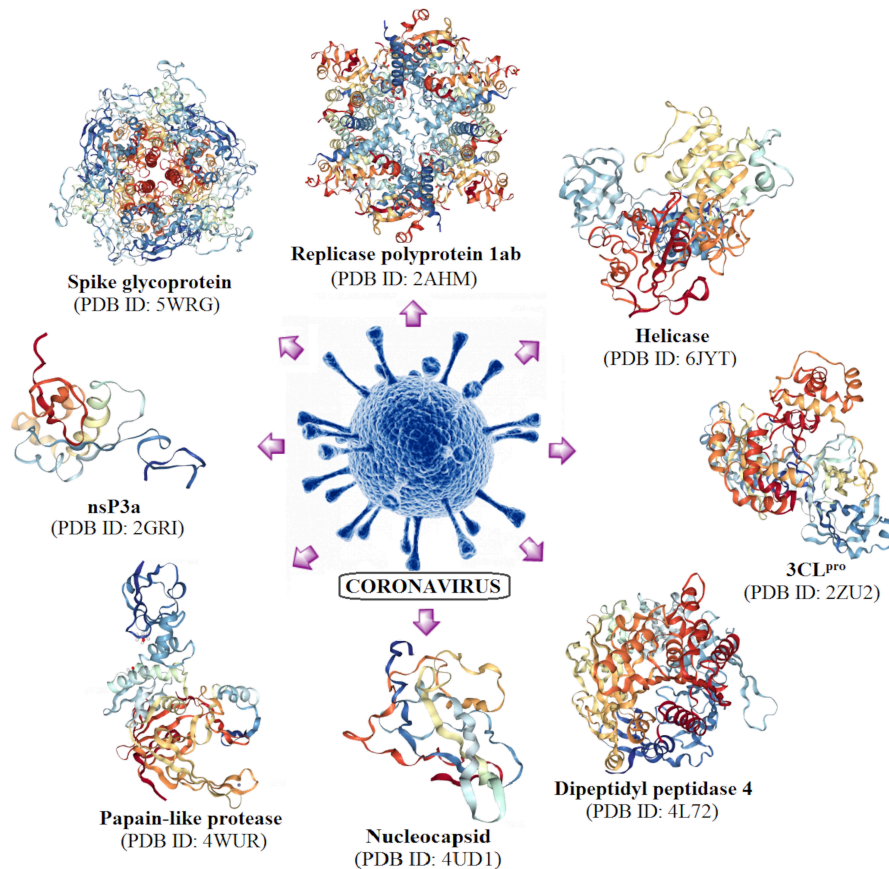
Despite considerable recent advances, there are no approved treatments or selective antiviral agents against HCoV, even after the first global 2002 SARS outbreak [17, 18]. Thus, current therapy includes supplemental oxygen and maintenance of body fluids. Moreover, hygienic precautions and mask utilization can reduce the risks of virus transmission [19 - 21].

Considering that a new therapy may take months or even years to become available, this chapter summarizes the methods and strategies used for discovering active molecules targeting SARS-CoV, MERS-CoV, and SARS-CoV-2. In this context, we aim to demonstrate the targets studied and the most active compounds, whether from synthetic or natural sources, to contribute to developing novel antiviral drugs that could be more selective and effective, reducing costs and time in the drug-race against this emerging global threat.

**BIOLOGICAL ASPECTS, SIGNS/SYMPTOMS AND DIAGNOSIS FOR MERS-COV, SARS-COV, AND SARS-COV-2**

Coronaviruses are known to cause severe respiratory, enteric, as well as systemic infection, which can affect humans, swine, camels, horses, cats, rodents, dogs, bats, among several other hosts, facilitating the global spread [22, 23].

Similarly, SARS-CoV, MERS-CoV, and SARS-CoV-2 encode structural proteins, such as spike glycoproteins (S protein), membrane (M protein), nucleocapsid (N protein), and envelope (E protein) proteins. Additionally, non-structural proteins, such as RNA-dependent RNA polymerase (RdRp), 3-chymotrypsin-like protease ( $3CL^{pro}$ ), helicase (Hel), and papain-like protease ( $PL^{pro}$ ) are promising targets for bioactive molecules that could lead to the development of an unprecedented drug against these severe infections (Fig. 1) [24, 25].



**Fig. (1).** Potential drug targets from Coronavirus.

## Opportunities Offered by Fragment-Based Drug Design in Antibiotic Development

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**Abstract:** In recent years, the discovery of new and effective antibiotics has slowed dramatically due to the rapid and widespread development of bacterial drug resistance and many pharmaceutical companies exiting the field. Reliance on conventional drug discovery methods, while effective in the past, has led to significant present-day challenges that are becoming increasingly difficult to overcome. A fundamental challenge to the development of new antibiotics against multi-drug resistant bacteria is the high cost of development relative to expected revenues. Fragment-based drug design (FBDD), which involves screening low molecular weight ligands, can help to drastically reduce the cost of finding initial hits compared with traditional high-throughput screening (HTS). In addition, a knowledge-driven and multi-pronged approach to the subsequent expansion of amenable fragments into high-affinity inhibitors may assist with overcoming hurdles in the hit-to-lead (H2L) optimisation process. Favourable pharmacological and physicochemical properties, as well as strategies against the development of resistance, can be incorporated as part of the fragment expansion process. This chapter discusses the features of the FBDD approach that are relevant and beneficial for antibiotic development. Successful examples and barriers to progress from hit discovery to H2L development, as well as patents, are presented. Finally, the outlook for FBDD in the field of antibiotic development, including the latest FBDD advances and challenges, is discussed.

**Keywords:** Antibiotic, Antibacterial, Antimicrobial, Drug design, Drug discovery, Drug lead, FBDD, FBLD, Fragment, Hit-to-lead, H2L, Resistance.

### INTRODUCTION

The discovery of antibiotics was one of the most significant medical achievements of the 20th century and has revolutionised modern medicine. However, their widespread overuse and misuse have resulted in the rise of antibacterial resistance

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across all drug classes. The emergence of multi-drug resistance in bacterial pathogens is of particular concern, specifically in Gram-negative bacteria which already have limited treatment options. International agencies such as the World Health Organization (WHO) have warned that unless urgent action is taken, the world may be about to enter a 'post-antibiotic' era, where once easily treatable infections will cause significant mortality or morbidity. It has been estimated that as many as 5–20 novel drugs would need to enter clinical development to keep pace with the high attrition rates of our current drug discovery model [1].

Despite these dire predictions, the last three decades have seen a distinct lack of discoveries of novel antibiotics. This can be partly attributed to the abandonment of antibiotic discovery methods by major pharmaceutical companies, for reasons that include the lack of revenue generated from competitive pricing, short-term use, the rapid rise of drug resistance and very high development costs. However, scientific challenges remain a fundamental cause of stagnation in the field [1].

High throughput screening (HTS) is a well-established and commonly used small-molecule drug discovery method employed by pharmaceutical companies. It has yielded the majority of the drugs in clinical use or undergoing clinical trials to date [2]. The fundamental requirement of HTS is the successful identification from the screening library of at least one compound with high potency (sub  $\mu\text{M}$  affinity; a "hit") that can progress through the hit-to-lead (H2L) development phase. HTS libraries typically need to contain  $> 10^5$  drug-like compounds ( $\sim 300\text{--}500$  Da) to cover enough structural scaffolds and functional chemical space to provide a reasonable chance of finding hits. As such, HTS is often a very expensive process and rarely affordable for academic researchers and smaller biotechnology companies. Despite the cost, a number of large pharmaceutical companies have attempted HTS for antibacterials but it has not been effective in bringing new antibiotics into the drug discovery pipeline. For example, between 1995 and 2001, GlaxoSmithKline (GSK) led a concerted HTS campaign during which 300 bacterial target genes were independently screened against over half a million compounds. However, only five potential leads were identified with all failing to progress to clinical trials due to a lack of broad-spectrum activity and drug-like properties [3]. This is by no means an exception and similar experiences have resulted in only a handful of large companies remaining engaged with antibiotic development today [4].

The successful revitalisation of the development of new antibiotics requires a concerted effort. To drive scientific breakthroughs, new research strategies must be explored, innovative government policies to change funding schemes implemented, and market subsidies to promote investment in the sector provided. An increasing number of policy statements and publications (such as reviewed by

Shlaes and Bradford [5]) have supported “fixing the broken antibiotic market” and some progress has been made. For example, there have been discussions in the United States about the possibility of funding antibiotic innovations with vouchers [6], as well as significant buy-ins from non-government charitable agencies. With this backdrop, it is ever more important that researchers, both from industry and academia, re-join the research and development effort to produce new antibiotics while utilising more time- and cost-efficient methodologies and tools to overcome the problems that have plagued the field. In this chapter, we first outline the potential merits of the fragment-based drug design (FBDD) approach for antibiotic development. Four case studies, including relevant patents, are then presented to showcase how FBDD has assisted to resolve some of the key scientific challenges in the antibiotic discovery pipeline. Finally, we discuss recent technical advances that can be incorporated into a FBDD research workflow to assist future antibiotic development efforts.

## **POTENTIAL ADVANTAGES OF FBDD FOR DEVELOPMENT OF ANTIBIOTICS**

Over the last two decades, FBDD has been established as a mainstream approach and a success story for developing new small molecule drugs for treating a range of conditions [7]. Despite this relatively short timeframe, FBDD has already led to 40+ lead molecules in clinical trials and four drugs on the market (Table 1, see <https://practicalfragments.blogspot.com/2020/3/fragments-in-clinic-2020-edition.html> for the latest update) [8]. In some cases, FBDD has yielded lead molecules where HTS has failed. This has given hope that FBDD may provide a system for the development of drugs against otherwise “impossible” targets and establish pathways for finding new antibiotics in the face of rising drug resistance.

A number of excellent reviews have recently described FBDD methodology and its advantages and caveats [9 - 11], from library design [12] to new and integrated screening methodologies [13], including a focus on specific protein classes such as kinases [14, 15]. Therefore, herein only a very brief summary of the technique is provided in order to introduce terms and abbreviations frequently used in the literature and in this chapter. Very briefly, an FBDD campaign begins with screening a library of low molecular weight compounds (termed fragments) which are only a fraction of the size of more typical drug-like molecules utilised in HTS. The small size of FBDD library compounds means only a few thousand fragments can effectively sample the chemical and structural space required to yield hits against a target. Compared to HTS, this makes FBDD more cost-effective and accessible to researchers. However, because of the small size of library fragments, the affinity of binding fragments for the target is typically very weak (hundreds of  $\mu\text{M}$  or even low mM). As such, FBDD relies on a plethora of techniques



## Phage Therapy as a Tool for Control of Foodborne Diseases: Advantages and Limitations

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**Abstract:** It is estimated that only in USA, foodborne pathogens cause 48 million illnesses, with 128,000 hospitalizations and 3,000 deaths each year. The growing global emergence of multi-drug-resistant infections raises the need to find alternative methods for the effective treatment of infectious illnesses. Phages possess properties that make them interesting but challenging candidates for different applications, including phage therapy against foodborne bacteria. The results of different clinical studies confirm the safety and efficiency of the use of bacteriophages for this purpose. Bacteriophage applications include water and food safety, agriculture and animal health. There are already several products available in the market. Studies indicate that phages have potent immunomodulatory and anti-inflammatory properties, and are recognized as an important part of the immune system. The use of bacteriophages for the control of foodborne infections should lead to promising alternative therapy. This review focuses on the application of bacteriophages as an antimicrobial alternative for therapies against antibiotic-resistant bacterial infections.

**Keywords:** Antibiotic-resistant bacteria, Antimicrobial, Bacteria, Bacteriophage, Biological control, Food, Foodborne pathogens, Foodborne diseases, Phage therapy, Safety food.

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## INTRODUCTION

Foodborne pathogens cause 48 million illnesses with 128,000 hospitalizations and 3000 deaths each year in the USA; 9.4 million of them are caused by 31 known pathogens of bacterial, viral, and parasitic origin. Around 800 annual outbreaks are reported, and the most frequent bacterial pathogens include *Campylobacter* sp., *Salmonella enterica* non-typhoid, *Clostridium botulinum*, *Escherichia coli*, and *Listeria monocytogenes* [1, 2].

Recently, some strategies have been explored to reduce foodborne bacterial disease incidence. The uncontrolled use of antibiotics in antimicrobial therapies brings a negative impact on human health. Improper use of antibiotics has promoted bacterial resistance that reduces the effectiveness of antibiotic treatments [3, 4]. The worldwide increase in multi-drug resistant bacteria is alarming. Bacteria that form biofilms are particularly resistant to antibiotics [5]. Thus, substantial efforts are made to produce antibiotics and non-antibiotic derivatives, such as vaccines, immunostimulants, adjuvants, and probiotics. However, the production of drugs is not enough to cover their global demand [6]. Finding alternative ways to treat infectious diseases is necessary. These factors have aroused interest in phage therapy around the world with clinical studies confirming the safe use of bacteriophages. This review focuses on the application of bacteriophages as an antimicrobial alternative for therapies against antibiotic-resistant bacterial infections.

## BACTERIOPHAGE

### History

The term bacteriophage was coined by the Canadian bacteriologist Felix d'Herelle in 1917, to describe both: the phenomenon of a spontaneous decrease in bacterial culture's turbidity and the presence of a hypothetical agent behind this process. d'Herelle believed in the presence of a virus or a small microbe that could pass through the best bacteriological filters [7]. The English bacteriologist Frederick Twort had described a similar phenomenon in 1915, observing the bactericidal effect of these viruses [8].

Moreover, in the 1920s, d'Herelle published extensive work on phage biology after performing numerous studies on the isolation, characterization, and clinical evaluation of them. d'Herelle proposed the existence of viruses capable of infecting bacteria. This work allowed him to start as an assistant at the International Institute of Bacteriophages in Georgia. During his studies, he made filtrates without bacteria from fecal specimens from patients suffering dysentery and mixed them and incubated with *Shigella* strains isolated from patients. In an

agar culture, d'Herelle observed the appearance of small and precise areas that contained no bacteria, which were later called plaques [7].

Shortly after the discovery, d'Herelle used phages to treat dysentery. Probably it was the first description of using bacteriophage as a therapeutic agent. The studies were performed in the *Hôpital des enfants-malades* in Paris in 1919 under the clinical supervision of Professor Victor-Henri Hutinel, pediatrics head of the hospital. d'Herelle, Hutinel and several hospital workers ingested the phage preparation to confirm its safety before administering it the next day to a 12-year-old child with severe dysentery. The symptoms of the patient ceased after a single administration of the phage, and the child recovered entirely in a few days [9]. The laboratory of d'Herelle in Paris produced at least five phage preparations against various bacterial infections that were marketed. Later it became the well-known French company L'Oréal [10].

Therapeutic phages were also produced in the United States. In 1940, Eli Lilly Company (Indianapolis, Indiana) produced seven phage products for human use, including targeted preparations against *Staphylococcus*, *Streptococcus*, *Escherichia coli*, and other bacterial pathogens. Initially, the bacteriophages seemed to offer great potential as first-line therapies against infectious diseases in the pre-antibiotic era. Until World War II, many countries used phage therapy [11]. In almost the entire western world, the production of phages as therapies ceased due to the arrival of antibiotics. However, in Eastern Europe and the former Soviet Union, phages continued to be therapeutically used altogether with or instead of other therapeutic medicines [11].

### **General Phage Characteristics**

Phages are the most abundant biological entities on Earth, estimated to be a total of  $10^{30}$  to  $10^{32}$  viral particles throughout the planet and play a decisive role in the balance of bacterial ecosystems [12]. It is thought that phages play a key ecological role in the modulation of ecological equilibria, in preventing bacterial population overgrowth in nature, and helps in the recycling of nutrients *via* lysis of bacterial cells in these environmental niches [13]. Additionally, prokaryotic evolution is mediated by phages through gene transfer processes, such as transduction and lysogenic conversion. Phages are present in soil, oceans and sediments, river, underground water, plants, animal biomass, waste streams from agricultural and food industries, households, human waste, hospitals, schools, airports and municipal sewage [14].

Bacteriophages are viruses that lack genomic attributes to generate ATP. Hence, they are obligate intracellular parasites, do not have a cytoplasmic membrane and are quite complex macromolecules of nucleic acids and proteins. They are inert in

## Subtractive Genomics Approaches: Towards Anti-Bacterial Drug Discovery

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**Abstract:** Pathogenic bacteria are evolving at a much faster rate and have the ability to acquire new antibacterial resistance patterns. The most common pathogenic bacteria are now becoming increasingly resistant to available antibiotics. The CDC has suggested to find alternative therapeutics to combat the growing antimicrobial resistance. Thanks to technological development in sequencing platforms and sophisticated bioinformatics pipelines, it is now easier to analyze large-scale genomic data and propose alternative and novel treatment options. Subtractive genomics is one such approach that mines whole genomic DNA for identification of potential drug target(s). This strategy employs various computational filters using databases and online servers to screen and prioritize certain candidate proteins. Each filter analyzes the whole proteome of bacteria under study in a step-wise manner. Initially, strain-specific paralogous and host-specific homologous sequences are subtracted from the bacterial proteome to remove duplicates and prevent cytotoxicity and autoimmunity related challenges. The sorted proteome is further refined to identify essential genes involved in crucial metabolic pathways of the pathogen and thus can be used as targets for treatment interventions. Functional annotation is carried out to elucidate the involvement of these proteins in important cellular processes, metabolic pathway, and subcellular location analyses are carried out for finding the probable cellular location of the candidate proteins in the cell. Proteins with certain physicochemical properties like favorable molecular weight, hydrophobicity, and pI are rendered fine drug targets, thus filter. Importantly, the scrutinized proteins are screened against FDA approved DrugBank to identify their druggability potential. Finally, molecular docking analyses of the novel druggable targets with already present drugs are carried out. Only then, the prioritized candidate proteins can prove to be promising candidates for novel drug design and development.

**Keywords:** Cytoplasmic proteins, Drug targets, Membrane proteins, Metabolic pathways, Proteome, Subtractive proteomics.

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## INTRODUCTION

Comparative and subtractive genomics approach, together with metabolic-pathway analysis provide a potent regime to categorize the set of proteins that are essential for the pathogen's existence but are not present in the host [1]. Subtracting host genome from the set of pathogen essential genes assists in finding non-human homologous protein candidates which guarantee no interaction of drugs with host proteins [2]. Subtraction in its literal terms means removed from bottom, taking a smaller yet important chunk from a larger dataset. Conversely, comparative genomics approach focuses on choosing proteins that are highly conserved among a number of species and interpreting them as promising targets. Subtractive genomics is an approach where the genomic dataset is analyzed and a smaller chunk under observation is subtracted and analyzed for its likely results. Using innovative bioinformatics tools that have been integrated with genomics, proteomics, and metabolomics may support the identification of putative drug targets against pathogens causing deadly infectious diseases [3]. After the target(s) have been predicted, *in silico* virtual screening of different chemical databases could generate fresh opportunities to choose and design the finest inhibitors [4].

### Characteristics of an Ideal Drug Target

A candidate that fulfils the following criteria can be referred to as an ideal drug target.

- i. It must be involved in the pathogenesis of the organism under study or significant for its survival.
- ii. It should be structurally druggable; possess the power to bind small molecules (inhibitors).
- iii. It should be functionally and structurally characterized to assist the study of small molecular inhibition via already established assays
- iv. It should be different from already known drug targets against which FDA have approved drugs, in order to escape the toxicity of cross-resistance [5].

Both wet lab and dry lab studies can assist drug target prediction, however, computational biology aided methods are preferred as they cut down cost, save time and resources.

### Significance of Drug Target Identification

For the past few years, Drug target discovery has been the fundamental emphasis in both the research and development sector as well as in the pharmaceutical

industry. Choosing the precise drug targets is required for the fruitful development, testing, and marketing of drugs [6].

Literature indicates that therapeutic drug targets belong to approximately 130 different protein families but mainly confine to G-protein-coupled receptors, enzymes, nuclear hormone receptors, certain transporters, as well as ion channels [7].

Prediction of putative targets in pathogens can significantly aid in developing innovative drug candidates against known and new targets or determining novel targets for existing drugs. Nevertheless, the experimental methods for this are very costly, laborious and challenging.

Late phase drug approval-failures are contributing to the increasing economic strain. This has pushed scientists to optimize their drug target identification regime to accelerate success rates.

Although it is essential to adjust drugs according to efficacy, pharmacokinetics, and toxicity, however, it should be ensured that they modulate the appropriate target, to begin with.

Unfortunately, several drugs fail to give the promising results either due of their poor efficacy or toxicity issues, while dealing with large populations. Undoubtedly, minor changes can lead to significant enhancements to already practiced schemes. However, drug candidate optimization cannot help with these problems if the prevalence of the target is low among the focused patient population. Alongside, the improved success rates of drug approval by regulatory authorities significantly rely upon picking the most promising target timely.

The best targets are the one strongly associated with the disease under study, having a well-established function in pathology plus they can be detected in high numbers/amount among the concerned patient population. Selecting the optimum target effectively curbs the anticipated disease pathway while leaving all other related pathways unaffected to produce minimal or no side effects.

### **Subtractive Genomics Based Efforts for Drug Target Identification**

Drug target prediction efforts are initiated at the end of the previous century, probably started with drug targets prediction in pathogens utilizing the 10 publicly available complete genome sequences [2]. To date, drug target identification using *in silico* regime has been quite successful as it has yielded a large number of targets against several pathogens. This process mostly involves comparative genomics and DEG is a key component of the entire scheme [8]. Nevertheless,

## Recent Advances in the Discovery of Antimicrobials through Metagenomics

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**Abstract:** Natural products obtained from the microbes have been reported as substitutes to contemporary drugs obtained from plants. With the increasing need for new therapies, new natural products are being explored using the traditional methods. As only a small fraction of microbes can be cultured in the laboratory, many microbes continue to remain unexplored for their ability to synthesize secondary metabolites. In the past few decades, the reduced cost of DNA sequencing and developments in computational tools have made the Metagenomic Approach effective and popular. Uncultured microbes can be studied through bioprospecting of the unexplored geographical niches. Moreover, Bioinformatics tools have enabled us to find the gene clusters that, in metagenomics, imply the real potential of finding novel open reading frames (ORFs). Screening of genomes for secondary metabolite-genes like non-ribosomal peptide synthases (NRPS) and polyketide synthases (PKS), has resulted in the discovery of new or previously known metabolites. Technological advancement and innovations in the culture-independent approach have allowed us to explore novel chemistries from environmental samples to identify the molecules of therapeutic value. This chapter will discuss the methods for identifying secondary metabolite genes from the genome, and the new approaches for functional metagenomic screening toward the discovery of antimicrobials. Moreover, insights into this approach will be provided to generate opportunities to explore natural products for combating the global demand for novel antibiotics.

**Keywords:** Antimicrobial, Bioinformatics, Bioprospecting, Metagenomics, Mining, Multi-Drug Resistance, Non-Ribosomal Peptide Synthases, Polyketide Synthases, Secondary Metabolite-Regulated Expression, Substrate-Induced Gene Expression.

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## INTRODUCTION

Infectious diseases are the primary cause of morbidity and mortality globally. Human intervention and massive usage of antimicrobial compounds have contributed to the progression of drug-resistance in microorganism [1, 2]. As a result, microorganisms are becoming resistant to multiple drugs, making the treatment difficult and expensive and are becoming a worldwide threat [3]. The multi-drug resistant (MDR) microbial species include *Acinetobacter*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae* [4]. Some reports have stated that *N. gonorrhoeae* is evolving and becoming resistant to broad-spectrum drugs cephalosporin and fluoroquinolones. It has also been classified as the priority pathogen by the world health organization (WHO) [5]. On the other hand, various synthetic medicines like aspirin, diclofenac, and Ibuprofen are readily available in the market and widely used for treating different diseases. However, their association with minor side-effects like headaches and back pain and severe side-effects like toxicity, breathlessness and haemorrhage are grave matters of concern. These challenges have caused a shift towards the exploration of natural products [6, 7].

Nature provides a generous niche for a large variety of medicinal plants, marine and terrestrial organisms and microbes, from which new antimicrobial agents can be obtained [8]. Different microbes produce a diverse variety of structurally different compounds. Such compounds obtained from microbes as penicillin, gentamicin, omegamycin, and streptomycin, have encouraged the discovery of newer and better compounds, that can act as sedatives, pain killers, heart stimulators, and show anti-cancer activity [9]. A definitive characteristic of any medicine, whether man-made or natural is that it should be effective, non-toxic, target-specific, non-mutagenic, non-irritant and stable [10]. These variations in chemical structures of compounds help us in developing new compounds as well as scaffolds, which can help us to meet the demand and need of new drugs for treating critical human diseases [11].

Culture-dependent approaches have enabled the discovery of new bioactive molecules but are limited by the fact that many microbes continue to remain unexplored and uncultivable [12]. As a result, a large variety of microbes is expected to stay elusive if we rely only on the traditional culture-dependent approach [13]. Thus, to have better insights into the microbes, metagenomics has emerged as a valuable tool to unearth most unexplored microbes. We can now understand the diverse biochemical pathways of uncultured microbes and surpass the limitations of culture-dependent approaches [14]. The approaches used in bioprospecting the metagenomes for useful genes or ORFs have been summarized



in Fig. (1). Proximally-located genes generally encode the enzymes involved in the biosynthesis of metabolites. These genes together form a cluster called the biosynthetic gene cluster (BGC). Metagenomics entails the construction of metagenomic libraries, their screening, and recognising these biosynthetic gene clusters [15]. The researchers using this approach, thereby, highlight the importance of new habitats in the exploration of untapped microbes that produce useful antimicrobial compounds having lesser side-effects [16, 17]. Mining of the metagenomes for BGCs has given us non-ribosomal peptide synthetases (NRPSs), polyketide synthetases (PKSs) and NRPS-PKS complexes through robust screening of the potent antimicrobial producers producing diverse antimicrobial compounds [18].

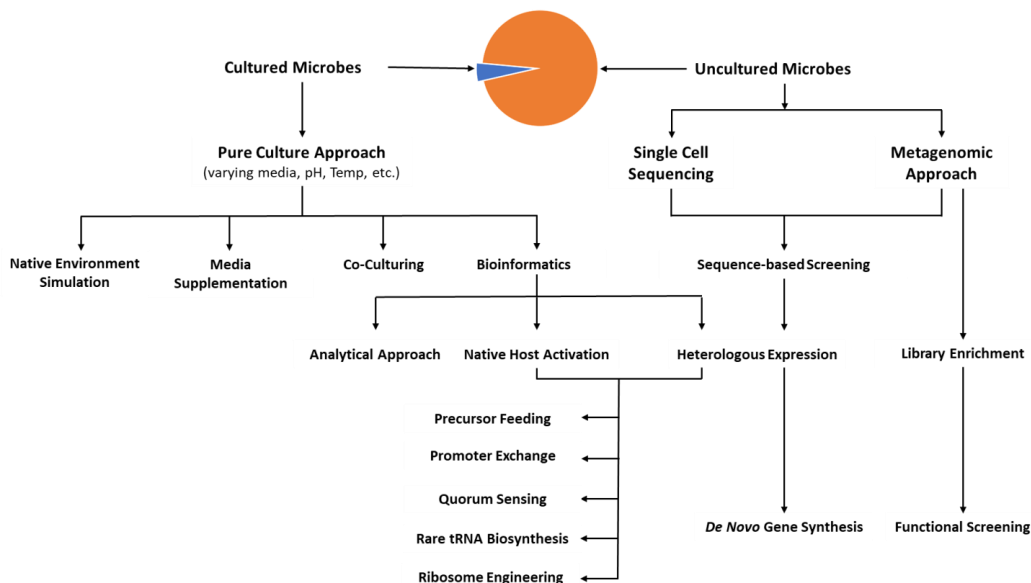


Fig. (1). Diagrammatic illustration of Approaches for Microbiome Mining.

## MAJOR MODULAR ENZYMES AND THEIR ASSOCIATED DOMAINS

Bacteria are the primary producers of secondary metabolites which exhibit antimicrobial activities. These compounds are synthesised by modular enzymes like NRPS and PKS [19]. The diversity of PKS and NRPS explains the diversity of secondary metabolites [20].

### Non-ribosomal Peptide Synthetases (NRPS)

Generally, the length of a non-ribosomal peptide is 2-45 amino acids, and their diversity is due to different combinations formed by amino acids, including *N*-

## Phyto-Nano-Antimicrobials: Synthesis, Characterization, Discovery, and Advances

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**Abstract:** Nanotechnology has brought a revolution to the world of science and medicine. With time, the dependency on nanotechnological advancement is increasing. Synthesis of nano-scale modulators is a significant domain of focus that employs crude formulations, retro-synthesized, and pure chemicals, mostly from herbal sources with lesser side effects. However, all these methods suffer from drawbacks and limitations. For an eco-friendly nanoparticle synthesis, green chemistry has evolved with a tangential approach for the synthesis of metals (Au, Ag) and metal oxides (ZnO, CuO, TiO). Green synthesis uses plant extracts (leaves, stem, shoot) and microbes (bacteria, fungi, yeast) as reducing intermediate for the production of nanoparticles.

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The advantage of these extracts lies within the phenolic constituents of aldehydes, ketones, proteins, and other biomolecules that implicate the reduction of the nanoparticles.

These green synthesized nanoparticles have high efficacy ranging from anti-bacterial, anti-fungal, and wide applications in medicine. In this chapter, we discuss the methods of green synthesis, their applications, and prospects. The current chapter will pave the way for future applications and better means for the synthesis of nanoparticles leading into a newer direction with varied recognition in nano-life sciences.

**Keywords:** Antibacterial, Antifungal, Gold, Green Synthesis, Metal oxides, Metal, Nanomaterials, Nanoparticles, Particle diameter, Particle size, Quantum dots, Silver.

## INTRODUCTION

Nanotechnology has changed the world with revolutionary thinking and methods that were not imaginable. This revolution has brought much new technology to the industry like CNT (Carbon nanotubes), QDs (Quantum dots), Graphene, and their composites. These inventions have a variety of uses and are involved in the day to day life. Nanotechnology has become an integral part of life, which is inseparable from us. To obtain nanomaterial of the desired shape, size, and functions, various fundamental synthesis approaches like top-down and bottom-up are used. To achieve this, methods like etching, milling, and sputtering are used. The use of the bottom-up approach has gained popularity in which nanomaterials are derived from simpler molecules. The methods used are chemical vapor deposition, Sol-gel processes, spray pyrolysis, laser pyrolysis, and atomic/molecular condensation. Chemical methods have a lot of limitations in getting the desired shape and size. These include environmental stability, lack of fundamental understanding of the mechanism, toxicity, extensive analysis, skilled operators, devices involved, and reduce/recycle/reuse. With the advancement of technology and the need for an eco-friendly approach, green synthesis of nanoparticles has revolutionized the world. This has caught attention in modern science and technology for nanomaterial synthesis. Green synthesis has an answer to many fundamental questions like minimization of waste, pollution reduction, and the use of safer solvent for synthesis. Green synthesis reduces the use of unwanted solvent, being sustainable and reliable. To achieve these objectives, a suitable solvent and a natural source are necessary. Green synthesis uses both plant extracts as well as microorganisms (fungi, bacteria, algae) for the production of metal/metal oxide nanoparticles. Green synthesis methods are dependent on parameters like pH, temperature, pressure, and solvent. For all these to be adequate, there is a need for plant extracts that can provide a holistic environment. Plant extracts are a rich source of aldehydes, ketones, flavanones, amines,

terpenoids, carboxylic acids, ascorbic acids, and phenols. These components help in the reduction of metals to metal oxide nanoparticles. This has a variety of uses ranging from antimicrobial to diagnostics. In this book chapter, an overview of types of green synthesis followed by their characterization technique is well explored. Further, the insights on nano-application with prospects have been elaborated exploiting the new avenues in nano-life sciences.

## **SYNTHESIS OF NANOPARTICLES**

Nanoparticles are mostly metal derived; the size ranges from 1-100 nanometers. Many novel approaches have been used for the synthesis of nanoparticles. Earlier synthetic approaches were common to derive nanoparticles but had pros and cons. Hence the green synthesis approach has given a new dimension to various studies. Nanoparticles can be produced using chemical synthesis, which requires high radiation, highly toxic reductants, and stabilizing agents, further they might harm both humans and the ecosystem. Nanoparticles have various processes and applications; for example, nanostructured powders by a flame pyrolysis process are used as commercial products, nanoparticle fabrication by precipitation, and surface controlling agents (SCAs), which have a role in size control and agglomeration avoidance. Standard nanoparticles that are chemically synthesized are SiO<sub>2</sub>, TiO<sub>2</sub>, FeOx, mainly used in *in vitro* tumor cell penetration and hyperthermal treatment [1]. Endosymbionts share a commensal relationship with the host organism. They have an application in nanoparticle synthesis; one among them is silver nanoparticles. A unique property of silver is that it is integrated into antimicrobial applications, biosensor materials, composite fibers, cryogenic super-conducting materials, cosmetic products, and electronic components. The silver nanoparticle was synthesized using endosymbionts *Pseudomonas fluorescens* CA 417 inhabiting *Coffea arabica* L. further characterized using spectroscopic techniques like UV-Vis spectroscopy at maximum absorption of 425nm. The average particle size was determined by dynamic light scattering (DLS) method that revealed the size to be 20.66 nm. These synthesized silver nanoparticles showed antibacterial activity on test pathogens. This also led to the feasibility study on both hydrophilic and hydrophobic substances [2].

To overcome all these problems, green synthesis of nanoparticles using plants and microorganisms makes it eco-friendly, cost-effective, with reduced use of organic solvent, averting the use of waste, and leading to reduction of pollution. “Green synthesis” aims to achieve the goal of eliminating hazardous substances if not entirely at least to the extent that it does not cause any harm to the environment by using ideal solvent systems and making the most use of natural resources. The synthesis of metallic nanoparticles through the biological precursor method depends on several factors such as pressure, temperature, solvent, and pH

## Aptamers as Anti-Infective Agents

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**Abstract:** Rapidly emerging drug resistance in all classes of pathogenic microorganisms has become a challenging task and a global health issue in recent years. There are very limited alternative options available to cure infectious diseases, as the rate of rise in drug resistance in infectious agents is higher than the arrival of new antimicrobial drugs. There is a dire need to look for new types of anti-infective agents, besides looking for new antibiotics. One of the promising types of antimicrobial agents is aptamers, synthesized through systematic evolution of ligands by exponential enrichment (SELEX) technique. Aptamers hold a significant promise for the treatment of various infectious diseases in the future. In the recent past, a number of successful attempts have been made to select and apply aptamers for the detection and binding of infectious agents and their products for therapeutic purposes. This chapter presents a basic introduction to aptamers and their application as anti-infective agents.

**Keywords:** Aptamers, Antibiotics, Drug Resistance, Infectious Diseases, Systemic Evolution of Ligands by Exponential Enrichment (SELEX).

### INTRODUCTION

Infectious diseases have been a major human enemy on the earth, accounting for millions of illnesses and deaths annually [1, 2]. Throughout human history, infectious diseases have been threatening the existence of the human race from extinction by global pandemics of major infectious diseases such as smallpox, typhoid, malaria, influenza, cholera and many others [3, 4]. The current global COVID-19 pandemic has emerged as one of the most notable global health crises across the globe, where billions of humans are staying at home as a measure to control the spread of disease and reduce morbidity and mortality rate [5].

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Combating infectious diseases has been a dream of scientists of all the time from the oldest civilization to modern age due to the seriousness of the issue [1]. The advent of antibiotics, vaccines, and several other classes of antimicrobial agents against infections caused by different kinds of microorganisms such as bacteria, fungi, viruses, protozoans as well as microbial toxins has played a key role in reducing the global burden of infectious diseases [6]. The novel concept of selective toxicity and magic bullets introduced by Paul Ehrlich in 1900 was found highly fascinating by the medical community and has been a milestone in the history of anti-infective drug discovery [7]. Soon, humans became capable of curing bacterial infectious diseases using a class of antimicrobial agents called *antibiotics*. Penicillin was the first antibiotic introduced by Sir Alexander Fleming in 1928 followed by a range of natural, semi-synthetic and synthetic antibiotics that made it possible to cure the diseases that humans seemed to be fighting throughout the known history [6].

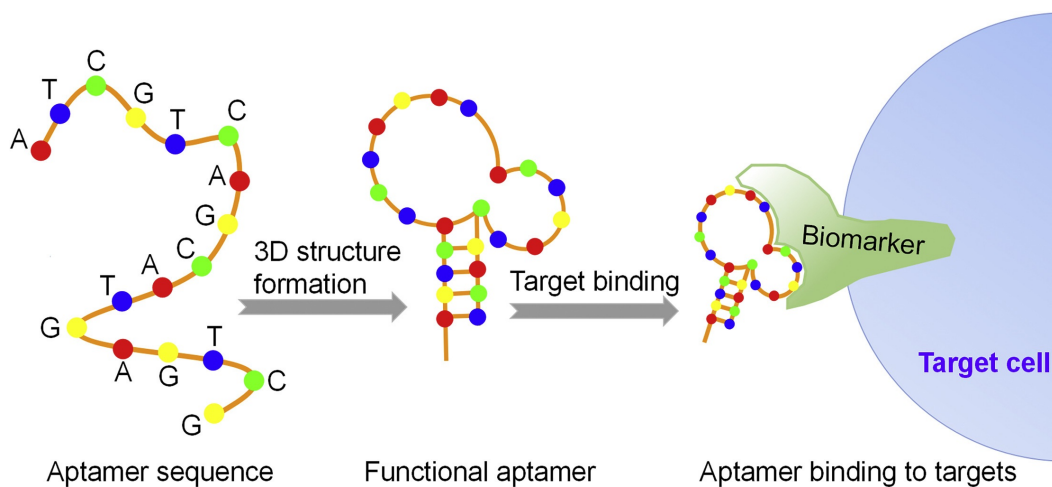
Drug resistance is a real challenge that humans are facing today, which is being reported in all classes of microorganisms [7, 8]. For instance, antibiotic resistance in bacteria is one of the most serious issue healthcare providers are facing today. The emergence of antibiotic resistant strains of pathogenic bacteria cost millions of lives across the globe annually. Multidrug resistant and extremely drug resistant clones of different bacterial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica* serovar. Typhi, *Mycobacterium tuberculosis*, *Acinetobacter baumannii* appear to be resistant to the majority of antibiotics. There are very few treatment options available in hand, as there are increasing reports of bacterial resistance to drugs of last resort such as vancomycin, colistin, carbapenems, *etc.* Similar situation is with other types of microorganisms such as viruses, protozoans and fungi [8 - 10].

On one hand, drug discovery efforts are aimed at focusing on new antibiotics, on the other hand, new options for the cure of infectious agents are being seriously considered. One of the alternative options is aptamers [9, 11]. In the last few decades, aptamers have attracted more attention due to their potential applications in therapeutics and diagnostics. This class of biomolecules is also being investigated for potential applications in the cure of infectious diseases [12, 13]. This chapter introduces aptamers, their unique features, methods of synthesis and their potential applications to target infectious agents.

## APTAMERS

The word aptamer is derived from a Latin word *aptus* meaning “To Fix”. Aptamers are single stranded oligonucleotides (ssDNA or RNA), or peptide sequences that bind their targets very specifically. Target may be may be a

biomolecule, toxin, cell, viral particle or even an inorganic substance [14]. These short secondary structures of ssDNA or RNA bind their target with high affinity and specificity [12, 15] (Fig. 1). Since early experiments, a high number of efforts have been made to select aptamers against a range of targets including different microbial species as well as their products. Attempts to study the binding of RNA sequences with proteins began in 1980s when scientists were studying interaction of human immunodeficiency syndrome virus (HIV) and adenovirus nucleic acids with proteins. It was discovered that these viruses produce small structural RNA sequences that bind viral or host cell proteins with high affinity and specificity [16, 17]. *In vitro* selection of aptamers against specific targets using systematic evolution of ligands by exponential enrichment (SELEX) was introduced by Szostak's and Gold's groups in 1990 [18, 19].



**Fig. (1).** Schematic diagram of aptamers binding its target. Oligonucleotide sequences first form 3-D structure that binds the target with higher affinity and specificity (Reproduced with permission from [100]).

As stated above, aptamers can bind a number of targets. Aptamer binding to their targets rely upon nature of their target as well as flexible nature of aptamers. The short oligonucleotide sequences of ssDNA or RNA aptamers can form a number of three dimensional structures, such as hairpin, pseudoknots, bulges and G-quadruplexes. On the basis of these conformations,

aptamers bind their targets *via* electrostatic interactions, hydrogen bonding, Van der Waals forces and  $\pi$ - $\pi$  stacking or combination some of these forces [20].

### SYSTEMIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICHMENT (SELEX)

RNA or DNA aptamers are selected randomly from a pool of oligonucleotide

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