Molecular Mechanism of Action of Antimicrobial Agents Against Clinically Important Human Pathogens: A Proteomics Approach

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Abstract

Globally, prevalence of infectious diseases profoundly affects the human health and economy. The failure of conventional antimicrobial agents and emergence of antimicrobial resistance among the pathogens are the major reason for spreading of infectious diseases. Hence, the need for novel therapy is increased to control infectious diseases. Deciphering the mode of action of drug is an important and crucial process in novel drug discovery. The understanding of antimicrobial resistance mechanisms in pathogens is a vital task during drug development. In these aspects, proteomics provides an innovative platform for the understanding of alteration in protein pathways that are associated with antimicrobial resistance in pathogens. Further, proteomics study is also supporting to recognize how the drug kills pathogen and also to reveal drug targeting pathways of pathogens. To develop more efficient and novel therapies against pathogen infections, it is essential to study the pathogen's response to drugs and establish resistance mechanisms in pathogens. Proteomics is most suitable tool to unveil molecular mechanism of antimicrobial agents. In this chapter, we aimed to reveal the significance of proteomics-based approaches in the identification of antimicrobial drug targets, to decipher the mechanism behind the drug resistance, and to unveil the mode of action of antimicrobial agents.

Keywords

Infectious disease · Antimicrobial agents · Antimicrobial resistance · Proteomics · Mode of action

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16.1 Introduction

Normally, human microbiome is harmless and also supporting the several biological processes of host. Likewise, several pathogens are also having the ability to survive in humans without causing any harmful infections till the host is with active immune system. However, immune system of host is compromised at certain circumstances, and infectious agents take advantage of this condition to cause diseases. Infectious diseases are majorly caused by microorganisms such as bacteria, fungi, and viruses [1, 2]. Among the infectious disease-causing pathogens, bacteria and fungi play an important role in causing infectious diseases to human [3, 4]. Bacteria possess the ability to cause mild to severe infections including soft tissue infections, urinary tract infections, bacteremia, tuberculosis, bacterial meningitis, wound infections, pneumonia, etc. [5].

Antibiotics are predominantly used to control the bacterial infections and it works by completely killing bacteria or by affecting bacterial growth and development. Since the origin of antibiotics, they have significantly reduced the impact of infectious diseases caused by bacteria. At the same time, inappropriate usage of antibiotics led to the development of antibiotics resistance among the bacterial species, and it reduces the efficacy of antibiotics against infectious diseases [6]. Same phenomenon was observed in treatment of other pathogens associated with infectious diseases such as fungi and protozoans [7]. Antimicrobial resistance is causing serious public health problem by increasing the hospitalization, treatment costs, morbidity, and mortality. According to recent data of the Centers for Disease Control and Prevention (CDC), yearly more than 2.8 million cases are observed with antibiotic-resistant infections, and 35,000 people are dying annually in the USA due to infectious disease caused by multidrug-resistant pathogens. Furthermore, the cost spent for the treatment of infectious disease immensely affected the economic productivity up to $1.5 billion per year in the USA. Overall, antimicrobial resistance impacted public health and economics worldwide [8–10]. Antimicrobial resistance is acquired by the pathogens through various mechanisms such as drug target modification, efflux pump, drug inactivation, biofilm formation, virulence factor secretion, etc. [11]. Therefore, emergence of antimicrobial resistance in pathogens is a global threat to human health and development. Hence, it requires more attention to develop novel antimicrobial therapy to treat infectious diseases caused by various pathogens.

In order to control antimicrobial resistance, numerous factors need to be considered during the development of novel antimicrobial agents including drug target, mechanism of action, drug efficacy, and safety. Among these factors, identification of drug targets and mechanism of action is a crucial aspect to avoid the development of antimicrobial resistance in pathogens. An important process in good drug designing is to identify the targets responsible for pathogen growth, development, and pathogenicity and designing drugs for vital druggable targets. The prediction of drug targets and mechanisms of action of drugs saves cost and time in the development of novel antimicrobial agents [11–14]. Elucidation of molecular mechanisms of drug has gained more importance for the development of new antimicrobial agents since it

reveals drug targeting pathways and thereby it supports the discovery of novel drug with multi-target potential against pathogens. Several approaches have been used to study drug targets and mechanism of action such as biochemical methods, computational methods, and omics-based methods including genomics-based method, deep sequencing method, and transcriptomics and proteomics approaches [15–17]. Among all these approaches, proteomics is the very effective method to identify drug target and molecular mechanism of action on infectious disease-causing pathogens. Since, proteins are real functional biomolecules in the living system, and they are involved in pathogenesis and resistance mechanism of pathogens [18–20]. In this regard, the current chapter briefs about importance of proteomics-based approaches to identify the molecular mechanism of action of antimicrobial agents against infectious disease-causing human pathogens.

16.2 Omics-Based Approaches to Analysis Mode of Action of Antimicrobial Agents

The omics-based approach is a true systematic approach to understand pathogen metabolism, drug resistance mechanism, and pathogenicity. Recently, omics-based approach is completely associated with drug discovery pipeline due to their role on drug target identification, drug target validation, druggable pathways, unraveling drug mode of actions, etc. [21, 22]. Genomics, transcriptomics, proteomics, and metabolomics are various omics strategies used in the novel drug discovery for microbial infections (Fig. 16.1) [23]. In genomics-based approach, whole genome sequencing helps antimicrobial drug discovery by exploring the virulence gene expression dynamism in pathogens grown in the absence and presence of novel antimicrobial agents of interest. And, it also reveals genes responsible for disruption of drug action, drug target modification, and efflux pump activations [24, 25]. Microarrays, RNA sequencing, and gene expression analyses belong to transcriptomics-based method to study the differential expression in organisms at various conditions. Transcriptomics analysis became an important method to understand mechanisms of pathogenicity and gene function, recognize new drug targets, and discover the drug action [26, 27]. Proteomics approach has attained more response in drug discovery due to limitations in the sensitivity and complexity of genomics and transcriptomics approaches. Proteomics techniques largely unveiled the proteins involved in pathogenesis, drug-resistant proteins, druggable targets, and pathways [28–30]. Proteomics-based approach plays unique and vital roles in the new drug development process because proteins are the real key players in living organisms.

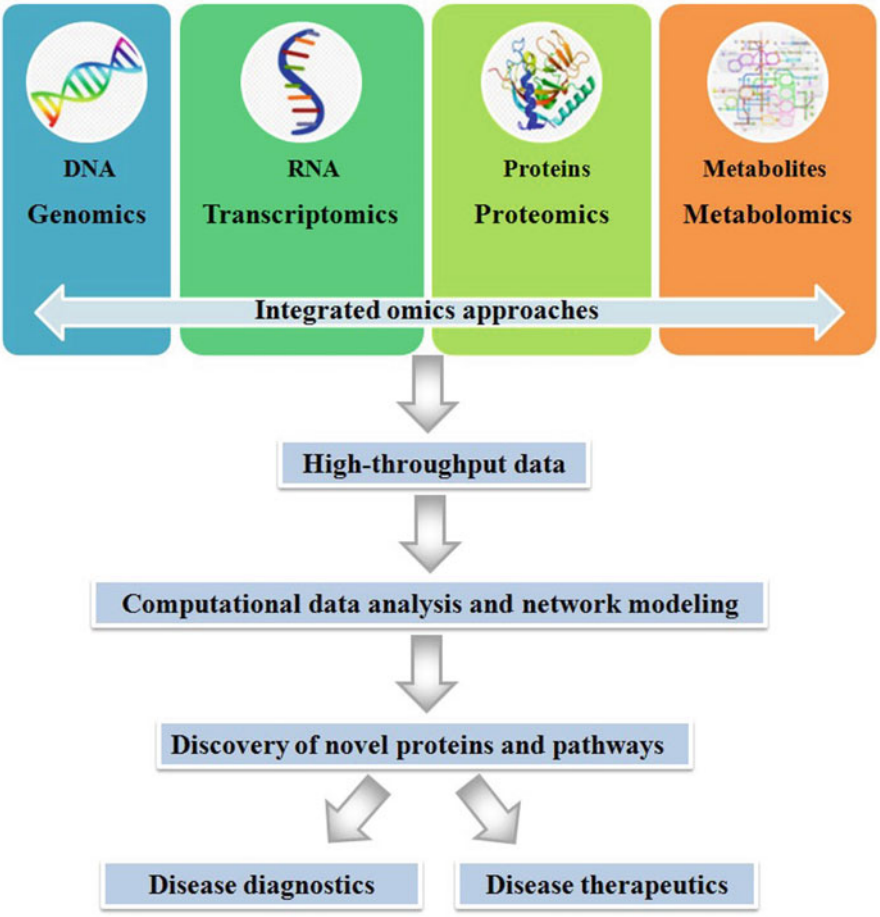

Fig. 16.1 Omics-based approaches in antimicrobial agent development

16.3 Proteomics and Its Significance

In 1994, Marc Wilkins coined the term proteome. Proteome is specifically studying the structures and functions of whole proteins of particular organisms. Proteins are important biomolecules of living organisms and are mediating all the metabolic pathways and biological process of organisms. Proteins are actual influencer in biological function and are not only dependent on DNA and mRNA expression levels but also with the posttranslational modification of host organisms [31–33]. Therefore, proteomics has been considered as the most suitable way to characterize biological systems when compared to genomics and transcriptomics. Proteomics is the technology used for the characterization, quantification, and identification of whole proteome of cell, tissue, or an organism. Proteomics-based approaches are

used in several aspects such as recognition of biomarkers, pathogenicity mechanisms, identification of differential expression of proteins, disease diagnosis, and elucidation of the role of proteins in various pathways of organisms [34, 35].

Proteomics technologies enable the identification of biomarkers for drug efficacy and toxicity, thereby helping the drug development process. Proteomics-based technologies are ideal choice for the identification of pathways targeted by novel drugs and revealed the function of proteins under disease conditions. It is useful to study the host-pathogen interactions, and thereby it supports to diagnosis of infectious diseases caused by pathogens. Proteomics experiments could be used for various purposes in clinical and health studies including monitoring the food proteins and biomarker discovery in various diseases such as tumor, AIDS, cardiovascular, and renal diseases [36–38]. Overall, the development and application of proteomics have been increased greatly in several promising new directions.

16.4 Proteomics in Drug Discovery

Currently, studying proteins of organism is an important process in drug discovery, and researchers have also focused on proteomics-based study to develop the novel drugs. Generally, application of proteomics includes identification and validation of drug target, identification of biomarkers to diagnose disease, assessment of toxicity of drugs, and mechanisms of action. Proteomics analyses reveal differential expression of proteins in response to infection-causing pathogens and thereby help to identify target proteins responsible for infections. These proteins could be a potential therapeutic target to design novel drugs [39–42].

Proteomics approaches also help to study the protein-protein interactions through which it supports to assess the impact of drugs on pathogens. Protein expression levels in pathogen are modified based on drug treatment and provide vital indications about drug effectiveness and targets. Target identification is the primary process in drug discovery, and also validation of identified targets is essential process in drug discovery pipeline [39, 40, 43–45]. Crucial process in drug development pipeline is to understand the virulence-associated pathways of microorganisms based on that drug need to be designed. Proteomics technologies are very useful to identify the pathways involved in pathogenesis of microorganisms. Proteomics-based technologies are suitable method to assess the drug resistance mechanisms in pathogens by comparing proteome of sensitive strains with resistance strains. Proteomics methods are also useful to identify posttranslational protein modifications including phosphorylation, glycosylation, acetylation, proteolysis, and amino acid polymorphisms in organisms [46–48]. On the whole, proteomics-based technologies are playing crucial role in terms of drug-target interaction, drug efficacy, drug toxicity, exposing the drug mechanism of action, drug resistance, etc.

16.5 Proteomics-Based Techniques for Studying Drug Development

Proteomics-based approaches have been emerged as a powerful tool to study mechanism of action of particular drug. Figure 16.2 illustrates the various methods used in proteomics-based approaches. The top-down proteomics assess the proteins and posttranslational modifications in the intact state. The bottom-up strategy is sensitive and powerful approach to examine multiple proteins in a single sample and majorly used in clinical diagnostics. In bottom-up proteomics, proteins are enzymatically digested into small peptides, whereas in middle-down approach, proteins are digested into large peptides. The peptide fingerprint of the proteins is identified using liquid chromatographic pre-fractionation followed by mass spectrometry. But in top-down proteomics approach, the complex protein mixtures are ionized, fragmented, and analyzed in the intact form to identify the targeted proteins of the respective organisms [49].

Various proteomics analytical methods have been used to assess the drug mechanisms of action such as gel-based and gel-free method. In gel-based, proteins are extracted from pathogens grown without and with drugs. Then, proteins are separated based on their isoelectric point and molecular weight using two-dimensional gel electrophoresis. Then, the differentially expressed protein spots on the gels are selected and identified with the help of mass spectrometry

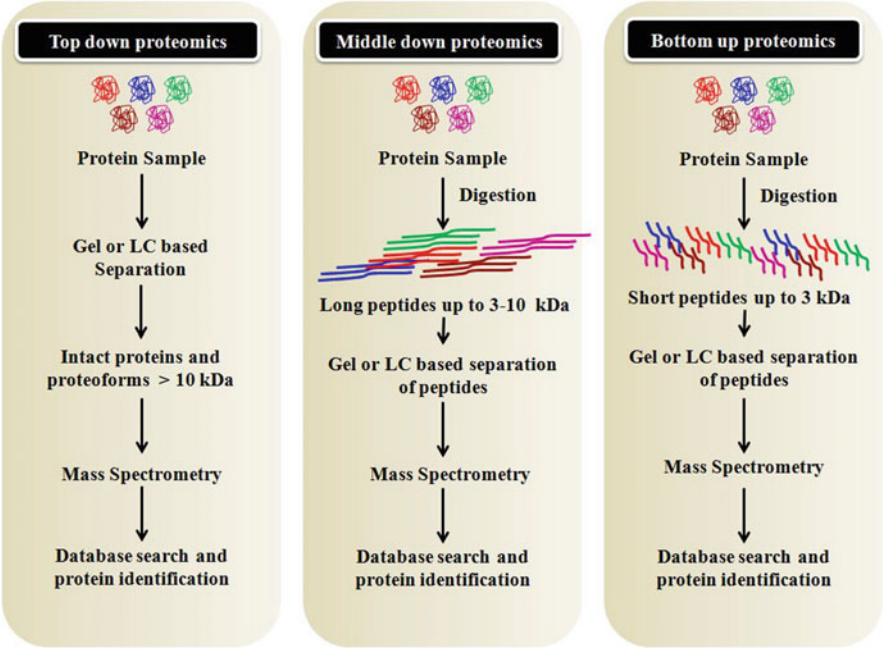

Fig. 16.2 Various strategies in proteomics-based approaches

analysis. On the other hand, in gel free methods, protein samples are subjected to mass spectrometric analysis without gel based separation. Various quantitative gel free proteomics technologies such as SILAC, iTRAQ and ICAT are widely used by researchers to identify differentially regulated proteins in crude samples [50–52]. Finally, differentially expressed proteins in drug-treated and untreated control proteins of organism could be found, and then drug-targeted pathways could be identified with computational analysis.

16.6 Infectious Disease-Causing Clinically Important Pathogens

Pathogen is defined as microorganism which possesses the ability to infect the host organisms. Mostly, human is an ideal choice for the pathogens because the nutritional availability and optimum temperature of the human body support the pathogen survival and multiplication. Numerous factors are responsible for spreading of infectious disease including global warming, urbanization, lifestyle, and inappropriate usage of antimicrobial agents [53–55]. Even though there is a remarkable advancement in the prevention, diagnosis, and treatment, infectious diseases still remain the leading cause of morbidity and mortality around the world. The resistance of pathogens to various antimicrobial agents has emerged as a major threat to the public health due to reduced efficacy of antimicrobial agents in the treatment of infectious diseases. Almost all the pathogens (bacteria, fungi, and virus) have high levels of multidrug resistance to conventional drugs. The development of drug resistance is a natural phenomenon due to inappropriate usage of antimicrobial drugs. Improper infection prevention and treatment led to emergence of drug resistance in pathogens [56–58]. Hence, it is more important to research more on the discovery of novel antimicrobial agent against infectious disease-causing pathogens.

Several studies have reported the various bacteria with high rates of infection such as *Acinetobacter* species, *.Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella*, *Shigella* species, *Neisseria*, and *Mycobacterium tuberculosis*. A high number of fungal infections are caused by *Trichosporon beigelii*, *Cryptococcus neoformans*, *Pseudallescheria boydii*, *Aspergillus* species, *Scopulariopsis* species, and *Candida* species. Prolonged viral infections have been observed in HIV virus, cytomegalovirus, herpes simplex virus, influenza A virus, varicella-zoster virus, hepatitis C, and SARS. *Plasmodium* species, *Toxoplasma gondii*, *Leishmania* species, *Trichomonas vaginalis*, and *Entamoeba* species are major disease-causing parasites [57, 59–61]. Table 16.1 presents the list of major infectious disease with causative agents.

Table 16.1 Major infectious diseases in humans

Type	Causative agent	Disease
Viral diseases	Influenza virus	Influenza
	Human immunodeficiency virus (HIV)	Acquired immunodeficiency syndrome (AIDS)
	Human papillomavirus (HPV)	Genital warts
	Hepatitis virus	Hepatitis
	Corona virus	Coronavirus disease (COVID)
Bacterial diseases	*Mycobacterium tuberculosis*	Tuberculosis
	Salmonella typhi	Typhoid
	Helicobacter pylori	Gastritis and ulcers
	Neisseria gonorrhoeae	Gonorrhea
	Neisseria meningitidis	Meningitis
	Staphylococcus aureus	Toxic shock syndrome and soft tissue skin infections
	Streptococcus pneumoniae	Pneumonia
	Streptococcus pyogenes	Scarlet fever and strep throat
	Clostridium tetani	Tetanus
	Corynebacterium diphtheriae	Diphtheria
	Vibrio cholerae	Cholera
Fungal diseases	*Candida* spp.	Candidiasis
	Cryptococcus neoformans	Cryptococcal meningitis
	Trichophyton, *Microsporum*, and *Epidermophyton* spp.	*Ringworm*
	Aspergillus spp.	Aspergillosis
	Blastomyces dermatitidis	Pulmonary blastomycosis
Protozoan diseases	*Plasmodium* spp.	Malaria
	Leishmania spp.	Leishmaniasis
	Trypanosoma spp.	African trypanosomiasis/African sleeping sickness
	Trypanosoma cruzi	Chagas disease/American trypanosomiasis
	Toxoplasma gondii	Toxoplasmosis

16.7 Mode of Action of Antimicrobial Agents Elucidated Through Proteomics-Based Approaches

For many decades, antimicrobial agents are used to eradicate the infectious diseases. However, these antimicrobial agents became ineffective against pathogens due to various mechanisms such as efflux pump, biofilm, virulence factors, alteration of drug targets, and drugs degradation [62–64]. Hence, to develop more proficient antimicrobial agents against infectious diseases, studying the mechanism of action of drugs is a primary process. Proteomics is an appropriate and powerful tool to study molecular response of pathogens to antimicrobial compounds. Analyzing the

pathogen response profiling in the presence of drug could reveal the mechanisms behind resistance and tolerance of pathogens to antimicrobial agents [29, 65]. In addition, another importance of proteomics analyses in drug discovery process is to verify the activity of particular drug by checking their efficacy with drug targets.

Recently, several studies have reported the mechanism of action of antimicrobial agents on pathogens and also validated the drug targets using proteomics-based approaches [66–68]. In previous studies, proteome of pathogens grown in the presence and absence of antimicrobial agent of interest is evaluated by assessing the changes in protein expression level using proteomics techniques. The previous study on *P. aeruginosa* identified that curcumin altered the expression of proteins involved in iron acquisition, pyoverdine and pyocyanin production in *P. aeruginosa* to inhibit biofilm and virulence factors [69]. Another proteomics-based study reported that the antibiofilm agent citral inhibited the biofilm and virulence of *S. aureus* by affecting the expression of IsaA, CodY, and SaeS [70]. Further, proteomics-based study unraveled the variation in ergosterol, sphingolipid, and oxidative stress systems in *Candida albicans* by antifungal agent myristic acid [71]. Various studies successfully explored the mechanism of action of identified drugs on pathogens using proteomics-based tools (Table 16.2).

16.8 Conclusion

The rise of antimicrobial resistance in microbial pathogens has become major problem in an international scale. Therefore, there is an everlasting need for the development of new antimicrobial agents and also need to increase their therapeutic potential through by understanding the drug molecular mechanisms of action. The scientific community started to reveal the drug actions on pathogens and also decipher the resistance and tolerance development to antimicrobial agents. The proteome approaches not only support the drug discovery but also improve novel strategies to infectious disease-causing pathogens. This chapter summarized the importance of proteomics-based approaches for understanding mode of action of antimicrobial agents, role of proteomics in drug discovery, and support of proteomics to develop novel treatment strategies to control infectious disease.

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Competing Interest
All the authors declare no conflict of interest.

Table 16.2 Mechanism of action of bioactives revealed by proteomics approaches

S. No.	Drug/compound	Target pathogen	Molecular mechanism	Reference
1.	Curcumin	*Pseudomonas aeruginosa*	Modulation of iron homeostasis and oxidative stress response	[69]
2.	Oleic acid	*Candida albicans*	Down regulation of ergosterol biosynthesis, lipase production, iron homeostasis	[66]
3.	Myristic acid	*Candida albicans*	Alteration of ergosterol, sphingolipid and oxidative stress	[71]
4.	Citral	*Acinetobacter baumannii*	Interruption of antibiotic resistance, antioxidant defense, and biofilm-associated two-component systems	[67]
5.	Vanillic acid	*Serratia marcescens*	S-layer, flagellin, and fatty acid biosynthesis	[68]
6.	Citral	*Staphylococcus aureus*	Modulation of pleotropic transcriptional repressor, cell wall homeostasis, exotoxin secretion	[70]
7.	3-p-Trans-coumaroyl-2-hydroxyquinic acid	*Staphylococcus aureus*	Disruption of cell membrane and peptidoglycan synthesis	[72]
8.	Bismuth drugs	*Helicobacter pylori*	Urease accessory protein ureg	[73]
9.	Chlorhexidine	*Acinetobacter baumannii*	Disruption of cell membrane	[74]
10.	Silver nanoparticles	*Pseudomonas aeruginosa*	Stimulation of oxidative stress response, an destroying iron homeostasis	[75]
11.	3-Hydroxyphenylacetic acid	*Pseudomonas aeruginosa*	Modulation of DNA replication and repair, RNA modifications	[76]
12.	Plantaricin gz1-27	*Staphylococcus aureus*	Modulation of biofilm formation, DNA replication and repair, and heat-shock	[77]
13.	Chitosan	*Escherichia coli*	Altering the stability of outer membrane	[78]
14.	Silver	*Escherichia coli*	Damage of multiple enzymes in glycolysis and tricarboxylic acid (tca) cycle	[79]
15.	Alpha-mangostin	*Staphylococcus epidermidis*	Alteration in cytoplasmic membrane integrity, cell division, teichoic acid biosynthesis	[80]

(continued)

Table 16.2 (continued)

S. No.	Drug/compound	Target pathogen	Molecular mechanism	Reference
16.	Daptomycin	*Staphylococcus aureus*	Disruption of cell membrane	[81]
17.	Rhodomyrtone	*Staphylococcus aureus*	Disruption of cell wall biosynthesis and cell division	[82]
18.	Gold nanoparticles	*Escherichia coli*	Modulation of energy metabolism and transcription	[83]
19.	Zinc oxide nanoparticle	*Acinetobacter baumannii*	Production of reactive oxygen species and membrane leakage	[84]
20.	B-hairpin macrocyclic peptide jb-95	*Escherichia coli*	Targeting outer membrane proteins	[85]

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