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REVIEW

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Abstract: Sequences of the genomes of all-important bacterial pathogens of man, plants, and animals have been completed. Still, it is not enough to achieve complete information of all the mechanisms controlling the biological processes of an organism. Along with all advances in different proteomics technologies, proteomics has completed our knowledge of biological processes all around the world. Proteomics is a valuable technique to explain the complement of proteins in any organism. One of the fields that has been notably benefited from other systems approaches is bacterial pathogenesis. An emerging field is to use proteomics to examine the infectious agents in terms of, among many, the response the host and pathogen to the infection process, which leads to a deeper knowledge of the mechanisms of bacterial virulence. This trend also enables us to identify quantitative measurements for proteins extracted from microorganisms. The present review study is an attempt to summarize a variety of different proteomic techniques and advances. The significant applications in bacterial pathogenesis studies are also covered. Moreover, the areas where proteomics may lead the future studies are introduced.

Keywords: bacterial pathogenesis studies, drug resistance, virulence, pathogen, proteomics

Introduction

Proteins are responsible for the biological functions that are dictated by genes in most cases.[1] The vast protein interaction networks control the strange cellular functions mainly. It is not possible to elaborate on these networks by merely relying on a single protein or a few proteins.[2] One of the ways to explain the biological systems of microorganisms in a large scale is proteomics. This technique provides us with information as to abundances, post-translational modifications, localization, interactions, and changes.[3] The sustained development of different proteomic technologies determines the capacity of proteomics to deal with major issues in the microbial field. There is a need for qualitative and quantitative studies in this field.[4] Other systems approaches have also notable benefits for microbial pathogenesis. There is an emerging trend of using proteomics to study infectious agents.[5] Using proteomic analysis to study protein profiles of bacterial pathogenesis is one of the main approaches to study proteins and interactions of the host-pathogen to find a deeper knowledge of dysregulations in infection disorders,[6] reveal bacterial resistance and virulence mechanisms,[7] and significant new targets for future drug discovery.[8] The immense potential of proteomic technologies to achieve a deeper insight into pathogenesis and develop therapeutic techniques is undeniable.

Pathogenic microorganisms like viruses, bacteria, or fungi[9] are responsible for infectious diseases and represent serious health risks for man, animals, and plants.[10] In spite of great works to develop new strategies to fight and prevent infections, the risk of newly emerging infectious diseases is undeniable.[11] The key point of infectious disease researches is a deeper insight into the functional interface between pathogenic microbes and their host cells.[12] Still, our knowledge of exact molecular adhesion, invasion, and replication is quite limited.[13] This lack of knowledge is an obstacle to develop new diagnostic and therapeutic strategies.[14] Additionally, the complicated interaction between host and pathogens is controlled by hundreds to thousands of proteins from both sides.[15] Most of the research work in this field has concentrated on determining the characterization of individual bacterial virulence factors and their interacting host targets using traditional genetic and biochemical approaches.[16] However, these studies fail to elaborate on the complicated multifactorial nature of host-pathogen interactions.[17] On the other hand, systems-level analyses give us a panoramic perspective of the functional host-pathogen interplay, which is significant improvement progress from the traditional reductionism-dominant research.[18] Therefore, transcriptomic studies have been around for several years and still, there is a great desire for measuring the final gene products, proteins. This is because of the poor correlation between mRNA and protein levels due to extensive post-transcriptional regulations.

One of the most important and interesting aspects of life is the ongoing interaction between hosts and pathogens.[19] These interactions take place throughout the long years of evolution; so that the hosts create defense mechanisms to handle pathogenic invasions and pathogens circumvent these new defense mechanisms.[20] Thanks to adaptation processes, some hosts can co-exist with or even have the benefit of pathogens. However, many pathogens still function as etiological agents for many life-threatening human diseases.[21] Therefore, having a clear understanding of host-pathogen interactions has led to the introduction of different means to prevent and treat infection-induced diseases. This study discusses the advantages and drawbacks of a gel-free/label-free proteomic technique along with introducing the potential application of proteomics in bacterial pathogen studies. In addition, the availability of proteomics approaches to uncover host-pathogen protein interaction networks, changes in the composition, and the organization of the host cell proteome are explained.

Applications of Proteomic Techniques in Bacteria

The metabolic aspects of an organism on a global scale are the subject matter of proteomic studies. Through this, large-scale proteomic technologies are developed prosperously.[5] Proteomic studies enable us to identify genome or/and measure proteins from microorganisms in a quantitative manner.[22] Researcher keeps developing proteomic techniques so that there are wide range methods and applications available.[23] Needless to say, proteomic technologies provide great potential to shed light on pathogenesis and develop new therapeutic techniques based on these insights. The latest studies have conducted reference proteomes for different bacterial pathogens and direct the future studies that need baseline proteomes for performing comparison.[24] Valuable information is provided by this technological platform as to signal transduction, adherence, and microbial-host interactions pertinent to bacterial pathogenesis.

Protein Identification

Measuring protein using the 2D gel electrophoresis method is the standard way for proteomic analysis.[25] The original separation technology (2-DE) can separate proteins based on their isoelectric point and molecular weight using SDS-polyacrylamide gel electrophoresis in the first and second dimensions, respectively.[26] In addition, to have sensitivity, covalent labeling of proteins with fluorescent Cy-dyes is used before separation. This technique is known as 2D difference gel electrophoresis (DIGE) can achieve higher quality and number of protein spots and gives more reliable gel matching.[27,28] On the other hand, these gel-based techniques are not sensitive enough to small quantities of proteins and they have limited proteome resolution.[29] Another disadvantage of these approaches is their poor performance in detecting different types of post-translational modifications of a single protein that causes crosstalk among signal pathways.[30] While one of the disadvantages of membrane proteomics based on the gel-free approach is the solubilization of membranous proteins, which is because of different optimum condition,[31] the volume of data available for membrane protein repertoire is growing.[32] Several bacterial studies including *Mycobacterium tuberculosis*,[33] *Scheffersomyces*

stipitis,[34] and *Staphylococcus aureus*[35] have used a gel-free technique, which further indicates the potential of this method by the identification of a far larger number of proteins. Gel-based and gel-free protein quantification, which are used as complementary approaches, are effective techniques to analyze the regulatory mechanisms utilized by bacteria. In *Klebsiella pneumoniae* as a successful example, the P13K-mediated vesicular transport was identified by the combination of both approaches.[36] Thus, it is essential for studies on plant stress responses to carefully select the proteomic approaches and cellular events that should be resolved by the approach.

Quantitative Proteomics

Both relative and absolute protein quantification are supported by mass spectrometry (MS)-based quantification strategy.[37] Metabolic in vivo labeling techniques like SILAC (stable isotope labeling with amino acids in cell culture) and[15] N labeling makes it possible to measure smaller measurement bias.[38] A chemical in vitro labeling methods like the ICAT (isotope-coded affinity tag),[18] O labeling, TMT (tandem mass tags) and iTRAQ (isobaric tags for relative and absolute quantification) can be used for static samples such as clinical samples.[39,40] Another identical strategy called isotope-coded protein label (ICPL) labels both N-termini and lysine side chains and is used at the protein level.[41] Currently, TMT and iTRAQ are the most commonly used techniques for labeling as it can be used for differential quantification of different protein post-translational modifications.[42] The iTRAQ-based differential proteomics of total proteins using a *Rhodococcus* sp. BAP-1induced by fluoranthene showed a decrease in the abundance of cytochrome ubiquinol oxidase subunit, NAD(P) transhydrogenase subunit alpha, 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase; still, there was an increase in the abundance of NADPH-dependent FMN reductase, 30S ribosomal protein S2, and S-ribosylhomocysteinase.[43–45] A technique to find differential bacterial proteomic profiling of *Staphylococcus aureus*[46] is the iTRAQ-based strategy; still, limitations of label-based techniques create problems in experimental design to compare samples so that only a few studies have used iTRAQ-based strategy in bacteria. These studies have been mainly on the stress response, which needs comparison among multiple conditions.[47] The use of the iTRAQ-based technique to subcellular compartments has the limitation of expensive reagents and the complicated process of preparing samples.[48] On the other hand, label-free quantitation is free

of any limitations as to the number of samples for analysis.[49,50] With label-free quantitation based on MS/MS, liquid chromatography (LC) is used to separate the digested peptides and transferred to a first mass spectrometer (MS1) where the chromatograms depicting signal intensities are obtained to measure the abundance of each peptide.[51] The peptide ions are adopted for deeper fragmentation in MS2 and determine the parent ion.[52] Label-free LC-MS/MS gives us the chance for wide quantification of proteins.[53] Thanks to advantages like easy sample preparation that is done faster in gel-free, label-free quantification allows accumulation of large volume of data in *S. aureus* proteomics, revealing central responses of *S. aureus* exposure to cold stress. In the case of subcellular proteomics in *S. aureus*, changes in the specific factors indicate the importance of citric acid-related signal transduction,[54] which controls the early stage of the bacteria's response to stress. Still, the unsolved problem in this method is how to optimize LC-MS chromatogram alignment for accurate quantification.[55] Many platforms use MS/MS scan times or base peak information to align chromatograms.[56] The merit of gel-free, label-free proteomics is in the ease of sample preparation and the acceptance for data production.[57] That to the large-scale data analysis of accumulated data on protein abundance[58] it is possible to elucidate biological processes that are missed in small-scale experiments (Table 1).

Proteomics Methods to Provide Mechanistic Insights in Infectious Diseases

Infection by different pathogens that are intrinsic to our ecosystem is the main reason for human disease and death worldwide.[59] An interesting aspect of life is the ongoing interaction between hosts and pathogens.[20] Researchers have concentrated on creating a molecular picture of pathogen infection and spread in an attempt to control the prevalence of infectious disease and develop better treatments for diseases.[60] Therefore, to find more about pathogen-host interactions is a driving force for the event of suggests that to stop and treat infection-induced diseases.[61] Over the past years, omic approaches have been introduced as effective tools in basic, translational, and clinical analysis to examine biological pathways effective in pathogen replication, host response, and disease progression. Proteomics tries to study the protein complement of biological systems and it has managed to show the discovery and

Table 1 Different Techniques in Quantitative Proteomic with the Associated Strengths and Limitations

Techniques	Methods	Strengths	Limitations	Representative References
2DE	Separation on a gel of the protein content of a sample in two dimensions according to mass and charge, gels are stained and spot intensities in samples are compared among different gels	Simple Robust Suitable for MS analysis	Involves large amount of sample Low throughput Poor recovery of hydrophobic proteins High inter-gel variability	[131]
2-DIGE	Measuring three samples per gels, each of them is labelled with a different fluorescent dye, and the intensities of each gel spot for each sample are measured at a wavelength specific for the label	Multiplexing Better quantitation Minimal gel to gel variation	Expensive Cy dyes Poor recovery of hydrophobic proteins Difficulty in separating low molecular weight compounds	[28]
Gel-free methods				
SILAC	Direct isotope labeling of cells Differential expression pattern A vital technique for secreted pathways and secreted proteins in cell culture Comprising labeling of the N-terminus and side chain amine groups of proteins	High throughput Robust Sensitive and simple	Only suitable for tissue culture models Costly reagents Not applicable to tissue sample	[38]
ICAT	Chemical isotope labeling for quantitative proteomics comparing relative protein abundance between two samples.	Selectively isolates peptide Compatible with any amount of protein Complexity of the peptide mixture is reduced	Post-translational modification information is frequently lost; cannot identify proteins with less than eight cysteines Large ICAT label (≈500 Da)	[39]
Protein microarrays	Binding of a targeted protein in one Sample to spotted probes on a "forward" microarray; conversely, binding of specific probes to a targeted protein in spotted samples on a "reverse" microarray; detection of bound proteins by direct labelling or by labelled secondary antibodies	Hight throughput Biomarker identity	Synthesis of many different probes Necessary; Identity or class of targeted proteins must be known Limited to detection of proteins targeted by the probes	[44]
ITRAQ	Isobaric tagging of peptides 4 or 8 analysis samples can be quantified Simultaneously; The method is based on the covalent labeling of the N-terminus and sidechain amines of peptides from protein digestions with tags of varying mass	Applicable to versatile samples Better quantitation Multiplexing	Expensive reagents Incomplete labeling Involves high amount of sample	[42]

(*Continued*)

Table 1 (Continued).

Techniques	Methods	Strengths	Limitations	Representative References
SELDI-TOF MS	Selected part of a protein mixture is Bound to a specific chromatographic surface and the rest washed away	High throughput Direct application of whole sample Small amount of starting material	Unsuitable for high molecular weight Proteins; Limited to detection of bound proteins; Lower resolution and mass accuracy	[51]
MALDI-TOF MS	Application of a protein mixture onto A gold plate; desorption of proteins from the plate by laser energy and measurement of the protein masses; comparison of peak intensities between multiple samples	High throughput	Need for sample fractionation of complex Samples; More starting material needed for sample fractionation; Unsuitable for high molecular weight proteins	[50]
LC-MS/MS	Separation of a mixture of peptides (resulting from protein digestion with trypsin) by one-, two- or three-dimensional LC and measurement of peptide masses by MS-MS	Direct identification of several hundred proteins per sample by MS-MS of peptides	Low throughput Time consuming Detection by MS-MS often not comprehensive; Complicating comparison of different samples	[53]
ICPL	After labeling of up to four different proteome states the samples can be combined and the complexity reduced by any separation method currently employed in protein chemistry	High-throughput quantitative proteome profiling on a global scale; able to detect to detect post-translational modifications and protein isoforms; applicable to Protein like tissue extracts or body fluids	Isotopic effect of deuterated tags interferes with retention time of the peptides	[41]
SRM	A powerful tandem mass spectrometry method that can be used to monitor target peptides within a complex protein digest with capability to multiplex the measurement of many analytes in parallel	Highly sensitive, quantitatively accurate and highly reproducible Quantification of post-translational modifications; Protein detection is relatively rapid Enables detection of non-abundant proteins	Detection and quantification of non- abundant proteins; Sensitivity is not comparable to immunological assays; Limited broad scale application because of difficulty in generating high-quality SRM assay	[45]
Label-free	Measuring the relative concentrations of peptide analytes within two or more samples; require the least sample preparation; as a tool to validate preliminary quantitative proteomics experiments	Avoids labeling Involves less amount of sample Higher proteome coverage	Not suitable for low abundant proteins Incomplete digestion may introduce error Multiplexed analysis not possible in one experiment; High throughput instrumentation	[49]

understanding of pathogen-host interactions.[6] This is the outcome of every improved proteomic technology that gives us sensitive protein detection and quantification tools. In addition, it increases awareness inside the biological science community and promotes using these approaches in innovative ways.

Intracellular Host-Pathogen Protein–Protein Interactions

The past ten years have witnessed a great contribution to comprehending host-pathogen interaction in the cellular life cycle of a pathogen by proteomic techniques.[62] Notably, the hyphenation of traditional analytical and biochemical techniques based on mass spectrometry has led to proteomic approaches that examine different aspects of the host-pathogen relationship.[11] Given that before reproducing to propagate, intracellular pathogens should pass through the host defenses, pathogen proteins interact with host proteins to either suppress or hijack the normal host protein functions.[6] Identification of those protein–protein interactions (PPIs) is essential, among many, for understanding the biology of infection; in addition, it can be used for new targets in treatments against human pathogens. In this study, the proteomics strategies that can be used to discover pathogen-host interaction networks, intact protein complexes, or direct interactions are reviewed. Furthermore, their strengths, limitations, and future promising directions in the context of finding out infectious diseases are discussed.

Building Host-Pathogen Protein Interaction Networks

Immunoaffinity purification along with mass spectrometry (IP-MS) are of the methods that have received the widest attention in pathogen-host interaction studies.[6] To isolate a protein in IP-MS, an antibody raised against the endogenous protein or epitope-tagging the protein of interest and using an antibody against that epitope are the options.[63] Therefore, the protein of interest and co-isolated interacting proteins are identified using MS. As to host-pathogen associations, the main advantages of IP-MS are the fact that experiments can be done in pertinent cellular model systems and the context of viral infection so that unbiased detection of PPIs is possible.[64] In the case of bacteria, IP-MS is utilized to detect interactions between effector proteins secreted by intracellular Salmonella and host proteins. Also, SILAC quantification is used to examine the specificity of interactions.[65] The multiplexing capability of TMT is not used in host-pathogen PPI studies yet; still, it allows for the simultaneous measurement of different infection time points along with negative controls to examine the specificity of the interactions detected.[20] Specific interactions of histone deacetylases by label-free methods and the relative stability of these interactions by SILAC were both determined using the combined analysis.[66] Thus, these approaches can be expanded to find valuable information as to dynamic host-pathogen interactions.

The fact that infections can cause significant changes in protein abundances in a cell and that the background of non-specific associations can differ completely from the one observed in an uninfected cell are key issues in pathogen-host interaction studies.[67] Thus, controlling isolations should be done in the same biological context under study. There are many computer algorithms available that utilize the data provided by control and experimental isolations to filter false-positive PPIs.[68] One of them is the significance analysis of interactome (SAINT).[69] This algorithm allocates interaction specificity scores to filter low-confidence interactions. Informatics approaches can also be employed to achieve a more refine identified interactions. For example, by creating extra controls for non-specific associations, like the contaminant repository for affinity purification (CRAPome).[70] A recently developed database for HSV-1 interactions, HVint, creates an integrated resource of HSV-1 protein interactions. It uses using evolutionary conservation of herpesvirus proteins to further predict additional interactions.[71] Thus, once a list of interactions is ready, these PPIs can be visualized within a functional network. This facilitates identifying the underlying biology in host-pathogen interactions. These results are indicative of the fact that further studies can improve the use of quantitative proteomics for comprehending infectious diseases.

Analysis of Intact Protein Complexes

To perform fully different functions, proteins usually exist simultaneously in distinct protein complexes. Though IP-MS gives us inventories of protein interactions, it averages together several protein complexes that host the same protein of interest.[72] Moreover, information about the ratio of associations in a complex is lost in the absence of fractionation and analysis. Top-down MS analyses where proteolytic digestion is not needed for analyzing proteins, can facilitate obtaining information about an intact macromolecule or multiprotein advanced.[73] Additionally, it protects each of the non-covalent interactions and consequently the post-translational state of the proteins inside the complex. Moreover, the technique is mostly used to individual infective agent proteins, like the hepatitis c virus pore protein p7,[74] and pathogenic complexes reconstituted in vitro (eg the Norwalk virus-like particles).[75] Still, top-down MS is not used to study host-pathogen complexes. Moreover, Top-down MS was

combined with ion mobility separation to find more about different forms of a multiprotein complex.[76] Therefore, top-down MS appears to be a reliable tool for studying host-pathogen protein complexes.

Detecting Direct Interactions

The yeast two-hybrid (Y2H) assay is one of the classic techniques for detecting direct PPIs.[77] The *Enterohemorrhagic E. coli* (EHEC) is not an intracellular pathogen; however, it has a close intracellular interaction with the host, as it injects 39 proteins into the host cytosol at least. The Y2H was also used to explain direct PPIs between EHEC and thus the human host cells.[78] A drawback of Y2H is that it has a relatively high false-positive rate, which is due to the non- physiological expression of proteins in cellular compartments where they are not commonly expressed. Moreover, because pathogen proteins are expressed beyond the context of an infection, many potentially relevant interactions might be missed. Along with MS, Hydrogen/deuterium exchange is another in vitro method to find the interacting regions of two proteins.[79] Besides, progresses made in search algorithms designed for cross-linking MS studies have added to their simple use.[80] Along with the identification of direct PPIs, crosslinkers are capable of stabilizing weaker or transient interactions and improving their identification; still, this increases non-specific associations. A study used those cross-linking tools and computational development to create a large dataset of direct interactions between human lung cells and *Acinetobacter baumannii*. Results have shown that a subset of that was useful for bacterial invasion.[81] Thereby, the examination of RNA-protein interactions by MS can improve our knowledge of post-transcriptional regulation processes that may have an important pathogenic infection.

Pathogen-Induced Proteome Alterations in Time and Space

A central role is played by the production, degradation, and spatial reorganization of proteins for the replication of pathogens.[82] Usually, the pathogen causes changes in the levels of specific host proteins required for replication. By global alterations in the proteome organization, the host also reacts to the pathogen invasion, which is critical for mounting effective defenses.[83] Thereby, these studies give us a deeper insight into the control of specific time points of infection and the required subcellular compartment reorganization.

Temporal Analysis of the Infected Cellular Proteome

Thanks to the provision of perfectly established protocols and the latest MS instrumentation, temporal proteome alterations are now accepted approaches.[84,85] A reliable was to characterize pathways controlled by the infectious agent and key protein effective in pathogenicity is temporal protein analyses.[85] Depended on cellular metabolism, viruses have attained several mechanisms such as controlling energy production and lipid synthesis.[86] Several studies have been performed on broad alterations in proteins metabolism regulation of human-relevant viruses, like the recently re-emerged Chikungunya virus,[87] human cytomegalovirus (HCMV),[88] flaviviruses,[89] and hepatitis C virus (HCV).[90] Additionally, some of these changes are temporally controlled; for instance, HCV regulation of glycolysis proteins happened only early in infection, whereas proteins used in lipid metabolism were increased continuously.[90] These proteome alterations are also capable of correlating with pathogenicity as it was reported by temporal proteomic studies on different influenza strains.[91] Notably, there is a relationship between regulation of specific proteins by the emerging and extremely virulent H7N9 influenza virus and its increased cytopathic effects.[92] Because infections cause a wide range of proteome alterations, further studies have focused on individual pathogenic proteins.

According to proteomic studies that introduce the RTA protein coded by Kaposi's sarcoma-associated virus (KHSV), which triggers lytic reactivation, known ARID3B as a number protein vital to initiate lytic replication.[93] Based on this knowledge, which was used by cell culture systems, temporal proteomic analyses of the infection process have been successfully used for in vivo studies in animal models challenged with viruses and bacterium.[94] Based on these findings, this technology makes it possible to carry out the in-depth characterization of specific organelles when infection appears so that there would be no need to eliminate the necessity of doing organelle enrichment and fractionation.

Spatial Cellular Proteome Organization During Infection

It is possible to determine infection-induced changes in protein abundances using proteome analyses on entire cells; however, the spatial information needed to understand proteome organization and characterize molecular mechanisms of pathogen infection is not provided.[95,96] To measure protein abundances in different parts of infected and clean cells we can tag cells by SILAC and

fractionated technique, which minimizes technical variability in the fractionation steps.[97] Another option is to keep the uninfected and infected samples separate throughout fractionation so that quantification can be done through label-free approaches or isobaric tags.[98] These alternatives bring the advantage of less limitation in the variety of samples so that analyzing multiple fractions and infection time points becomes possible. Changes that are induced by infection on the cell surface proteome prove the dynamic role of the plasma membrane proteome in the transport of metabolites with the extracellular space,[100] intracellular and living thing signaling,[99] and cell attachment during infection.[101] According to proteomic studies, viral-induced alterations play a role in the mitochondria biogenesis, oxidative phosphorylation, and the electron transport chain in return.[102] By integrating quantitative proteomics and live-cell microscopy, the present study introduces a wide range of alterations in organelle composition and form and distinct protein translocations between secretory organelles needed for the production of infectious particles are mentioned.[88] Moreover, the integration of strategies to follow the dynamic localization of proteins inside the cell[103] gives us more information about the spatial reorganization of the cell proteome when an infection takes place.

Pathogen-Induced Regulation of Protein Post-Translational Modifications

By altering protein interactions, stability, activity, and subcellular localization, post-translational modifications (PTMs) controls protein functions. Thereby, PTM regulation has a key role in the progression and results of infection on either host or pathogen proteins.[104] Cellular landscape studies on PTMs and their pathogen-induced regulation have yielded valuable insights into host-pathogen interactions.

Diverse Forms of Post-Translational Modifications are Relevant in the Context of Infection

Different PTMs are efficient means of controlling signal transduction, virulence and regulatory processes on bacterial proteins like phosphorylation, acetylation, methylation, and deamidation.[105] The PTMs are a key process in the life cycle of bacteria so that they can modulate main virulence factors and they are attractive targets for novel therapies.[106] Finding these PTMs in bacteria is a technical challenge as they are not easy to discover given that the modifications usually exist at low levels of abundance.[107]

To compensate this, specific enrichment strategies that target certain PTMs are used to lower peptide complexity and increase the chance of finding and characterizing; for instance, immunoaffinity enrichment is a standard way for lysine-acetylated peptides.[108] In addition, identical enrichment strategies are used to find phosphorylation events on serine, threonine, and tyrosine (S/T/Y) amino acid residues.[109]

Novel lysine-acetylation events in virulence factors help host immune response evasions like chitin-binding protein, a serine protease, exotoxin A, and hemolysin. This means that lysine acetylation events in *Pseudomonas aeruginosa* affect the mechanisms pertinent to virulence.[110] Results have shown that cysteine phosphorylation in *S. aureus* help in controlling bacterial virulence and vancomycin resistance.[111] The authors used high-resolution MS to explain in a site-specific fashion, that cysteine phosphorylation events took place in different proteins so that many of them are global regulators that control important biological processes.

MS as a Tool to Study Host and Pathogen Protein PTMs

Post-translational modifications can be observed in cells, and many of them are dynamically regulated when an infection occurs. Therefore, global PTM analyses can be done using proteomic methods.[112] Selected global PTM mapping is concentrated on specific types of modifications and it has been done for various pathogenic agents such as bacteria,[112] fungi,[113] protozoa,[114] and viruses[115] to detect and measure SUMOylations, phosphorylations, acetylations, and histone modifications.[116] The main tool for PTM discovery experiments is the selective enrichment of specific proteins or PTMs and then identifying the modified peptides.[117] Normally, this enrichment is done by antibodies against the PTM or protein or by a resin that can enrich a class of PTMs using the chemical properties.[118] Along with these discovery-driven experiments, targeted MS/MS methods including selected reaction monitoring (SRM) or parallel reaction monitoring (PRM) are tools for sensitive monitoring of PTMs on proteins of interest.[119] Despite their well-recognized value that makes accurate quantification of low abundance PTMs possible,[120] pathogen infection studies have not used these approaches frequently; still, they can be used more commonly in the future to widen our knowledge of proteome regulation during infection. Moreover, there is a lack of systematic examination of different types of

PTMs and regulation of them as to time and space in infectious contexts.[121] This is true for PTMs that are critical regulators of protein functions like phosphorylation, ubiquitination, and acetylation along with emerging PTMs of which our knowledge about their impact on protein functions like malonylation, succinylation, and lipoylation is limited.[122] Moreover, for the identified PTMs, the detailed effect of many of these modifications either in uninfected or infected cells is unclear.

Multi-Omics Integration for the Study of Host-Pathogen Interactions

There are several uses for Multi-omic approaches like determining the coding capability of pathogens, identifying key virulence factors, and outlining the responsibilities of the host to pathogenic infection.[123] Proteomics is also added to transcriptomic analyses to have a better annotation of infectious agent genomes,[124] provide experimental proof for genes, delineate intergenic events, and purify the limits of available gene models of pathogens.[125] Although, it is not easy to analyze the data of these varieties of experiments, there are procedural platforms to facilitate future proteogenomic analysis in pathogens.[126] Proteomics, glycopeptidomics, and glycomics were used to find glycosylation sites and glycoform distribution in different influenza strains.[127] This approach has enabled us to determine the glycosylation patterns of selective pressure obligatory by host immune factors, that influence the strain antigenicity and virulence.

While new omics methods are being introduced every day, it is important to integrate them with alternative omics approaches to achieve higher levels of data that might improve pathogenic research, like as integrating host and infectious agent PTMs[128] or subcellular location data.[88] A key point in multi-omics studies is that access to informatics platforms that may be accustomed to access and visualize the data such as Immunet.[129] Thereby, providing these resources is essential for generating data-driven hypotheses for future pathogenic. While IP-MS is designed for studying protein complexes within bacteria,[130] its use has remained limited to study in vivo pathogen-host cell protein interactions and their dynamic regulation throughout infection.[131] Proteomic approaches that support protein microarrays, complement IP-MS approaches and demonstrate interesting opportunities for high-throughput screening of infectious agent interactions. By recognizing protein-encoding plasmid DNA and then

translating it into exploitation noncellular expression systems merely before using the sample, the nucleic acid Programmable Protein Array (NAPPA) technology outperforms the common pitfalls that influence microarrays imprinted with purified proteins.[132] When used together, there would be no need to use antibodies or generate recombinant pathogenic strains. This can be specifically advantageous for basic analysis investigation into the molecular networks of infectious agent interactions.

Proteomics Methods to Provide Mechanistic Insights in Bacterial Antibiotic Resistance

Human health is growingly threatened by bacterial pathogens as the number and distribution of antibiotic-resistant bacterium and the rate of discovery of recent antimicrobials dwindles is increasing.[133] Since using antibiotics to fight infectious diseases, microorganisms have started to fight back. Using resistance mechanisms microbes can bypass and survive the action of antibiotic drugs.[134] There are several strategies to find these mechanisms and associated in-progress efforts to lower the steady increase in the number of treatment failures due to multi-drug-resistant microbes.[135] Proteomics is one of the key tools in this area of research. They have key roles in realizing the molecular mechanisms of bacterial pathogenesis and in distinctive disease outcome determinants.[136] The physical associations find by proteomics lead to tools to develop pathogen-specific treatment strategies that lower the spread of antibiotic resistance.[137] After the recent fast advances in whole-genome sequencing, proteomic technologies are used extensively to examine microbial gene expression.[138] Therefore, proteomics has emerged as a reliable tool to review bacteria. There are many comparative proteomic studies on bacteria-resistant to develop different antibiotics and some are mentioned in the following sections (Figure 1; Table 2).

Cell Wall-Acting Antibiotics
Beta-Lactams
Beta-lactams antibiotics are generally categorized as penicillin, cephalosporin, carbapenems, monobactam, beta-lactamase inhibitors, and other minor categories.[139] The beta-lactams halt the synthesis and/or stability of the cell envelope, which results in the biogenesis of cell-wall and loss of selective permeability and osmotic integrity in return and finally bacterial cell death.[140] Beta-lactam antibiotics
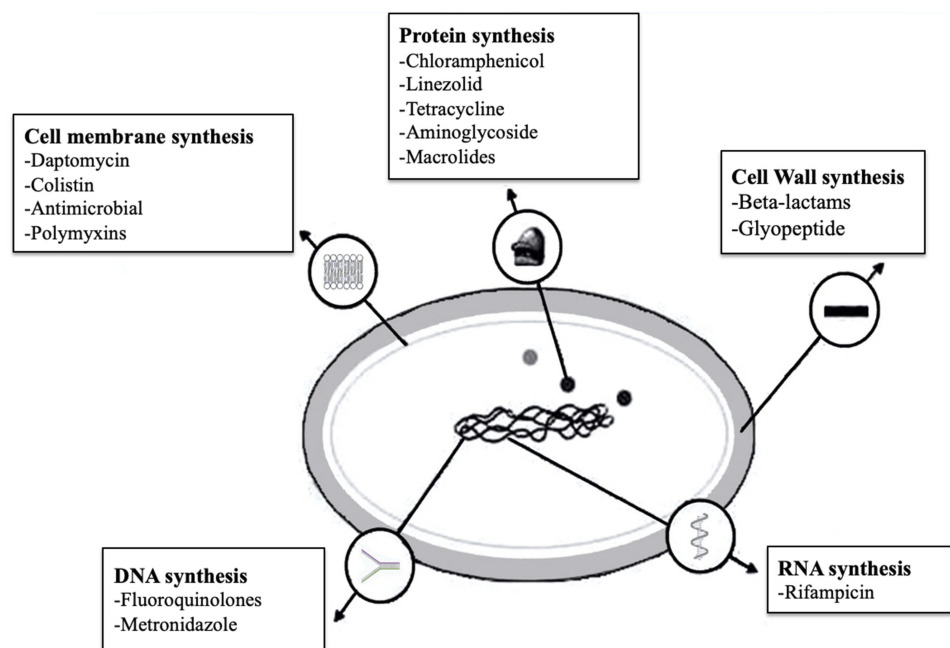
Figure 1 Overview of bacterial antibiotic resistance mechanisms. Antibiotics target essential bacterial processes and structures to inhibit cell growth and/or causing cell death. The major cellular targets for antibiotics include DNA replication (eg, fluoroquinolones), protein synthesis (eg aminoglycosides), cell wall integrity (eg, penicillins) and folic acid metabolism (eg, sulfonamides).

resistance is one of the commonly studied resistance based on proteomics methods.[141] Antibiotic hydrolyzing proteins is the main resistance mechanisms to beta-lactam antibiotics, which is also known as beta-lactamases.[142] There are other major mechanisms like imbalance in transport proteins such as efflux pumps and porins and alteration in the penicillin-binding protein targets.[143] The growing trend of using antibiotics has resulted in the rate of some key resistance strains like methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, and extended-spectrum beta-lactamase (ESBL),[144] as well as carbapenemase-producing *Enterobacteriaceae, Pseudomonas aeruginosa*, and *Acinetobacter baummanni*.[145] The findings by proteomic researchers give us deep insights into ampicillin-resistant *Pseudomonas aeruginosa*, where novel porins are involved in resistance.[146] Studies on the resistance to piperacillin/tazobactam in *Escherichia coli* have shown that the expression of porin OmpX was lowered and the expression of TolC increased.[147] Wither regard to the penicillin-tolerant Gram-positive *Streptococcus pyogenes*, overexpression of murein metabolism proteins and general alteration of bacterial physiology are reported.[148] Studies on methicillin-resistant *S. aureus* have revealed changes in cell physiology and overexpression of catalase and superoxide dismutase.[149] Alanine dehydrogenase has been found effective in antibiotic resistance.[150] Studies on inner membrane fraction

of carbapenem-resistant *A. baumanni* have revealed a relationship with beta-lactamase AmpC and OXA-51 production along with metabolic enzymes, elongation factor Tu, and ribosomal proteins.[151]

Glycopeptide

Glycopeptide vancomycin functions through stopping peptidoglycan synthesis. It binds to the DAla-DAla terminus of the nascent peptidoglycan and therefore blocks the correct synthesis.[152] Substitution of the DAla residue from peptidoglycan termini by D-lactose or D-Serine, in *Enterococcus* spp., was found to be the key mechanism of resistance to vancomycin.[153] In addition, in *S. aureus*, a more complicated scenario was proposed with diverse enzymes and gene clusters implicated in vancomycin-resistance. Resistant strains like vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant *enterococci* (VRE) are of main clinical concern.[154] Wang et al[155] studied vancomycin-resistant *Enterococcus faecalis* and investigated a reference strain (V583) and a clinical isolate (V309) with and without vancomycin. The results supported the regulation of the proteins involved in vancomycin resistance functions, virulence factors, stress, metabolism, translation, and conjunction. Ramos et al[156] determined the proteomic profiles of vancomycin-resistant *E. faecium* SU18 strain treated and not

Table 2 Proteomic Studies of Bacterial Antibiotic Resistance Mechanisms

Antibiotic	Pathogens	Physiological effects	Proteome Analysis	Representative References
Cell wall				
Vancomycin	*Enterococcus faecium*	Vancomycin resistance proteins increased; metabolism-related proteins decreased	2-DE and LC-MS/MS	[156]
Piperacillin/ tazobactam	*Escherichia coli*	Bacterial virulence, antibiotic resistance, DNA protection, and multidrug efflux pump expression associated with resistance	2D-flurorescence difference gel and electrophoresis (2D-DIGE)	[147]
Carbapenem	*Acinetobacter baumannii*	Beta-lactamases, energy, and protein production enzymes are upregulated; OmpW and surface antigen downregulated	2D-DIGE	[151]
Penicillin	*Streptococcus pyogenes*	Growth phase, stress, and fatty acid biosynthesis (FAB) proteins expression altered	Two dimensional gel electrophoresis (2-DE) and tandem mass spectrometry	[148]
Cell membrane				
Colistin	*Escherichia coli*	Outer membrane proteins, chaperones, protein biosynthesis factors and metabolic enzymes	2-DE and LC-MS/MS	[167]
Daptomycin	*Staphylococcus aureus*	Differences in biofilm formation proteins, cell wall-associated targets	iTRAQ and IPG-isoelectric focusing with LC-MS	[161]
Protein synthesis				
Kanamycin	*Escherichia coli*	Outer membrane protein expression altered. Identification of novel membrane MipA protein involved in antibiotic resistance	2-DE and LC-MS/MS	[197]
Tetracycline	*Acinetobacter baumannii*	Outer membrane proteins decreased expression in membrane and increased secretion	2-DE/MS-MS and 1-DE/LC/MS-MS	[190]
Linezolid	*Streptococcus pneumoniae*	Metabolism and transport of carbohydrates involved in resistance to linezolid	2-DE and iTRAQ	[187]
Protein synthesis				
Chloramphenicol	*Burkholderia thailandensis*	Overexpression of efflux pump systems associated with resistance	SDS-PAGE electrophoresis and LC-MS/MS	[183]
Erythromycin	*Streptococcus pneumoniae*	Glyceraldehyde-3-phosphate dehydrogenase upregulation in resistant strain	2-DE and LC-MS/MS	[201]
DNA synthesis				
Fluoroquinolones	*Pseudomonas aeruginosa*	Overexpression of ATP-binding component of ATP binding cassette (ABC)	2-DE and LC-MS/MS	[206]
Metronidazole	*Clostridium difficile*	RecA, ferric uptake regulator (Fur), putative nitro reductases and altered expression of stress-related proteins	iTRAQ and 2D-LC-MS/MS	[212]
RNA synthesis				
Rifampicin	*Brucella abortus*	Alterations in several metabolic processes and secretion mechanisms	2-DE and LC-MS/MS	[217]

treated with vancomycin. 14 proteins are differentially expressed in SU18. Proteins that played a role in the vancomycin resistance mechanisms demonstrated an increase in the presence of vancomycin; while there was a decrease in metabolism-related proteins, which results in compensatory effects. Notably, the proteomic profile of a group of heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) vancomycin susceptible *S. aureus* has been compared.[157] At first, five upregulated proteins in hVISA were detected and only one of them supported by real-time quantitative reverse transcription PCR (qRT-PCR) – ie the protein encoded by the isaA gene involved in cell wall biogenesis.

Cell Membrane-Acting Antibiotics
Daptomycin

A new mechanism of action that is demonstrated by daptomycin is a cyclic lipopeptide antibiotic.[158] This agent functions on the cell wall membrane structure and it is synthesized through binding to the cell membrane using a calcium-dependent mechanism. This results in the efflux of potassium ions of the bacterial cells.[159] This process results in bacterial cell death in.[160] Daptomycin is active against Gram-positive bacteria and it is clinically used to treat intense infections by these organisms (MRSA bacteremia, skin and soft tissue infections, endocarditis, and VRE infections,).[7] Based on comparative proteomics profiles in the daptomycin-susceptible *S. aureus* strain and the daptomycin-resistant *S. aureus* strain 701, there is a differential abundance of proteins in different functional categories, such as cell wall-associated targets and biofilm formation proteins.[161] In addition, LiaI and LiaH proteins caused (429-fold) by daptomycin, using the proteomic approach of a daptomycin-susceptible *B. subtilis* strain W168 in presence of daptomycin treatment of sublethal amount (1 μg/mL).[162] The removal of the response regulator LiaR controls the expression of liaIH in daptomycin-resistant *E. faecalis* and reversed resistance to daptomycin. This leads to hypersusceptibility to daptomycin.[163] Thereby, it can be concluded that LiaR is the main regulator that protects cell membranes against diverse antimicrobial agents, by regulating the expression of different genes like liaH gene. Thus, the study showed that several proteins of different functional categories, including cell wall-associated targets, had different expressions.

Colistin

As an antimicrobial peptide, Colistin interacts with the bacterial outer membrane, by replacing bacterial counter ions in the lipopolysaccharide (LPS).[164] Hydrophobic and hydrophilic regions interact with the cytoplasmic membrane as a detergent and make the membrane solubilized.[165] The main common mechanisms of resistance to colistin are modifications to LPS.[166] Li et al[167] studied proteins in mcr-1-mediated colistin-resistant and -susceptible *Escherichia coli* to achieve a deeper insight into the colistin resistance mechanism. They showed that the substrate phosphoethanolamine (PEA) for mcr-1 that mediated colistin resistance was accumulated in colistin-resistant *E. coli*. It is notable that along with PEA modification of the bacterial cell membrane lipid A, mcr-1 has an effect on the biosynthesis and transport of lipoprotein in colistin resistance through disrupting the expression of efflux pump proteins that play a role in the resistance pathway of cationic antimicrobial peptide (CAMP). There is an association between the low intracellular c-di-GMP level in dispersed cells of a *P. aeruginosa* strain and a higher abundance of proteins required by the virulence and development of antimicrobial peptide resistance in *P. aeruginosa*.[168] Therefore, *P. aeruginosa* cells with low c-di-GMP levels act as an extra immunity to colistin than *P. aeruginosa* cells with high c-di-GMP levels.

Antimicrobial

A polypeptide known as antimicrobial peptides (AMPs) is generated endogenously to defend the host against microbial invasion. Also, they function actively against a wide range of microorganisms such as MDR bacteria.[169] The bacterium, in Vibrio parahaemolyticus, reacts to AMPs by up-regulating the efflux channel, increasing the energy consumption performance, repairing damaged membranes effectively, and down-regulating of carbohydrate and nucleotide metabolism to preserve energy.[170] In the case of Mycoplasma pulmonis, we know that the activation of the stress response, which also triggers mutations in the hrcA gene, can improve the development of resistance to AMPs like melittin or gramicidin D.[171] Furthermore, 2-DE analyses, in *M. pulmonis*, indicated the up-regulation of enzymes playing a role in energy metabolism as a feasible outcome of the increased energy demand of the resistant strains.[172] Proteins that are effective in Vibrio parahaemolyticus AMP resistance were indicated by Shen et al.[173] In addition, subculture of *V.parahaemolyticus* strains exposed to four different AMPs demonstrated resistant strains.

Additionally, two OMPs (TolC, flagellin) and five IMPs (transcription termination factor NusA, EF-Tu, ATP synthase α subunit, dihydrolipoamide dehydrogenase, long-chain FA transport protein, FadL) were spotted by analyses, which had changed the expression between the WT and AMP-resistant strain significantly. Moreover, it is believed that up-regulation of the energy-dependent MDR efflux transporter (TolC and F1-ATPa), repair of damaged membranes effectively (DLD) and AMPs cellular penetration (down-regulation of FadL)[174] mediate AMP resistance. These findings showed that the upregulation of the TolC pump is a form of probable resistance mechanism described with different antibiotics.

Polymyxins

As well-established antibiotics, Polymyxins have lately drawn a great deal of attention as a result of the growing incidence of infections caused by multidrug-resistant Gram-negative bacteria.[175] The polymyxins that are produced by *Bacillus polymyxa* are a set of cyclic polypeptides that altering the permeability of the cytoplasmic membrane[176] to induce their effect. Based on MALDI-TOF analysis of the lipid A extracted from RamA-overexpressing strains of *K. pneumoniae*, RamA increases colistin/polymyxin resistance levels.[177] This increase was done by RamA that is directly bound to lipid A biosynthesis genes like *lpxC* that modifies the structure of lipid A. A study showed that overexpression of a *pagL*-specific sRNA, Sr006 increased *pagL* mRNA, lipid A deacetylation, and polymyxin B resistance in *P. aeruginosa*. It also revealed that a *pagL* knockout led to a decrease in polymyxin B resistance.[178] Thus, the fact that *PagL* is upregulated in chlorhexidine-resistant *P. aeruginosa* means that the resistance action mechanism to chlorhexidine might be the same, partially, as of polymyxins.

Protein Synthesis-Acting Antibiotics
Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic that plays a role in the synthesis of mitochondrial protein.[179] The chloramphenicol functions through creating bounds to the 50S bacterial ribosomal subunit and inhibiting the synthesis of protein.[142] Studies have explained resistance to chloramphenicol as part of the presence of the chloramphenicol acetyltransferase (CAT), which is an enzyme that inactivates the drug.[180] Li et al[181] found six outer membrane proteins and one protein of the location was unknown and in charge of chloramphenicol (CAP)-resistant Escherichia coli and for survival in medium with suddenly strong CAP treatment. The study argued that 4 out of the 7 proteins, including OmpC, TolC, OmpT, and OmpW, were notably changed and they could be considered as potential targets for developing new medicines against CAP-resistant E. coli. Therefore, antibodies that acted against the known OM proteins were utilized to show antibody-combating bacterial growth.[182] As the results showed anti-TolC had highly significant inhibition on bacterial growth in medium with CAP. This highlights a potential novel method to treat infection by antibiotic-resistant bacteria. Antibiotic resistance mechanisms *Burkholderia thailandensis* were used to examine SDS-PAGE coupled with LC nanoelectrospray MS/MS.[183] The resistance induced by the chloramphenicol was effective with structurally unrelated antibiotics such as quinolones and tetracyclines.[184] In general, the results showed that there was an association between the multi-drug resistance phenotype, found in chloramphenicol-resistant variants and the over-expression of two different efflux pumps, which were able to expel antibiotics from several families.

Linezolid

One of the oxazolidinone antibiotics for clinical treatment of severe infections with resistance against Gram-positive bacteria is Linezolid.[185] Linezolid an oxazolidinone that binds to the 23S rRNA (Ribosomal ribonucleic acid) and it demonstrates different resistance mechanisms such as a higher expression of ABC transporters, mutations in 23S rRNA, mutations in ribosomal proteins L3 and L4, and mutations in an RNA methyltransferase.[7] Voigt et al[186] studied expressions of the protein in *S. aureus* after a short exposure to MCB3681, a new quinolonyl-oxazolidinone antibacterial. They tried to answer the question if MCB3681 can influence the expression of proteins different from those influenced by ciprofloxacin or linezolid. Their findings indicated that the effect of MCB3681 on the proteome signature of treated *S. aureus* cells was not the same as ciprofloxacin or linezolid. Proteomic and transcriptomic screening of linezolid indicated that it is feasible to increase the metabolism and transport of carbohydrates in like linezolid-resistant *S. pneumoniae* mutants.[187] That is, resistant strains overexpressed several glycolytic proteins, enzymes, and transporters involved in sugar metabolism.

Tetracycline

Aaminoacyl tRNA binding to the mRNA-ribosome complex can be inhibited by tetracycline.[188] There are at least three mechanisms that create cell resistance to tetracycline including enzymatic inactivation of tetracycline, efflux, and ribosomal protection.[189] Yun et al[190] utilized proteomic techniques to examine the surface proteome of *A. baumannii* DU202 outer membrane vesicles (OMV). This surface is notable resistant to tetracycline, after imipenem treatment. They reported a higher OMV secretion after exposure to imipenem treatment and an increase cytotoxicity towards A549 human lung carcinoma cells. The differential proteome of *E. coli* K12 BW25113 exposed to chlortetracycline stress was labeled using isobaric tags and quantitative proteomics technology for relative and absolute quantitation of the labeling (Lin et al[191]). The role of ribosome protein complexes in the translation process was improved in general in the presence of chlortetracycline stress, which is a compensatory mechanism created by the chlortetracycline effect on the ribosome. Therefore, these findings give us deeper insights to hypothesize the role of energy to guarantees cell survival. It appears that they change their metabolism to achieve a basic level of energy production and ensure their survival in the presence of the stress caused by a harmful antibiotic agent. This hypothesis can be the subject to future studies on proteomics.

Aminoglycoside

Through blocking the small 16S subunit of the bacterial ribosome, aminoglycoside antibiotic family can stop protein synthesis.[192] We know three aminoglycoside resistance mechanisms including lowered uptake or decreased cell permeability, modification at the ribosomal binding sites, and generation of aminoglycoside modifying enzymes.[193] Low levels of NarG and NarH and two elements of respiratory nitrate reductase (Nar) were found in streptomycin, gentamicin, ceftazidime, tetracycline, and nalidixic acid-resistant *E. coli* strains in a proteomic study based on native/SDS-PAGE.[194] The protein expression profiles of a high-level spectinomycin-resistant (clinical isolate) and a susceptible (reference strain) *Neisseria gonorrhoeae* treated by sub minimal inhibitory concentrations (subMICs) of spectinomycin were compared by Nabu et al.[195] Both strains demonstrated overexpression of 50S ribosomal protein L7/L12 which is a key element for ribosomal translocation. This means that compensatory mechanisms function might be in response to antibiotics

that inhibit protein synthesis. To create the effects of gentamicin on the proteomes of aerobic and oxygen-limited *E. coli*, Proteomics techniques are an options.[196] In addition, protein involvement in kanamycin resistance was reported in a proteomic and Western blotting study of the *E. coli* K-12 outer membrane (OM). Zhang et al[197] reported an increase of some OM proteins like Tolc, TsX, and OstA, and a decrease of MipA, OmpA, FadL, and OmpW OM proteins in the kanamycin-resistant *E. coli* K-12 strain. They argued that MipA is a new OM protein implicated in antibiotic resistance.

Macrolides

Macrolide antibiotics function through creating a reversible bound to the P site on the subunit 23 S of the bacterial ribosome.[198] The main tool of bacterial to resist against macrolides is through post-transcriptional methylation of the 23S bacterial ribosomal RNA.[199] Among experimental types of acquired resistance are a generation of drug-inactivating enzymes (esterases or kinases) and generation of active ATP (Adenosine triphosphate)-dependent efflux proteins that transport the drug outside of the cell.[200] Cash et al[201] studies the proteins synthesized by erythromycin-susceptible and erythromycin-resistant *S. pneumoniae* using peptide mass mapping to find a 38500 Dalton protein upregulated in resistant strains as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Assuming that this a probable reason for the resistance against erythromycin, the authors maintained that was an increase in energy production for the efflux system. Smiley et al[202] conducted a proteomic study on isolated sarcosine- insoluble outer membrane protein (OMP) fractions obtained from clarithromycin-susceptible and resistant *Helicobacter pylori* strains. They demonstrated a decrease in iron-regulated membrane protein, UreaseB, EF-Tu, and putative OMP; and an increase in the HopT (BabB) transmembrane protein, HofC, and OMP31 in clarithromycin-resistant *H. pylori*. These findings indicate changing the outer membrane protein profile can be considered as a new mechanism effective in clarithromycin resistance in *H. pylori*.

DNA Synthesis-Acting Antibiotics
Fluoroquinolones

A commonly used family of quinolones in clinical settings is Fluoroquinolones.[203] Quinolones inhibit the essential bacterial enzymes DNA gyrase and DNA topoisomerase IV.[204] There are three quinolones resistance mechanism namely mutations that change the drug targets, mutations that lower

drug accumulation and plasmids that defend cells against the lethal effects of quinolones.[205] Proteomic studies on protein expression levels have found 43 proteins with higher expression in *Salmonella enterica* serovar Typhimurium strains when a fluoroquinolone is added to the bacterial culture.[206] This means that the majority of these proteins were only a physiological reaction to fluoroquinolone; still, there was an association between the identified over-expressed AcrAB/TolC efflux pump and resistance. Proteomic analyses are conducted to examine the mechanisms at the protein level that confer resistance to fluoroquinolones. A comparison between the proteomes of fluoroquinolone-susceptible *Coxiella burnetii* and fluoroquinolone-resistant samples of the bacterium was done by Vranakis et al.[207] They showed diverse expressions of 15 bacterial proteins that had a role in different cellular processes, which indicate the multifaceted feature of the antibiotic resistance mechanism in the bacterium. Additionally, Lin et al[208] showed an increase in the OM proteins TolC, OmpT, OmpC, and OmpW and a decrease in FadL in the nalidixic acid-resistant *E. coli* strains. Generally, TolC and OmpC can have a stronger role in controlling nalidixic acid resistance comparing with the other identified outer membrane proteins.

Metronidazole

To inhibit nucleic acid synthesis, Metronidazole, as an antibiotic of the nitroimidazole class, disrupts the DNA of microbial cells.[209] A study on the protein profiles of a derivative of *Helicobacter pylori* strain 26695, featured with resistance to moderate levels of metronidazole, showed that the mutant strain improved the production of the resistant phenotype of different isoforms of alkyl hydroperoxide reductase when exposed to metronidazole.[210] A study on a metronidazole-resistant strain derived from *B. fragilis* ATCC 25285 indicated that the proteomic changes influenced a wide range of metabolic proteins such as lactate dehydrogenase and flavodoxin.[147] Changes in the metabolic pathway effective in pyruvate-ferredoxin oxidoreductase has been also reported by a multidisciplinary analysis of a non-toxigenic *Clostridium difficile* strain that was resistive to metronidazole.[211] Moreover, according to proteomic analysis, DNA repair proteins, putative nitroreductases and the ferric uptake regulator are regulated in a NAP1 *C. difficile* clinical isolate that is resistive to metronidazole.[212] The results mean that there can be an association between a multi-factorial response and high-level metronidazole-resistance in *C. difficile*, such as the probable roles of altered iron metabolism and/or DNA repair.

RNA Synthesis-Acting Antibiotics
Rifampicin

By inhibiting bacterial DNA-dependent RNA polymerase, rifampicin can inhibit bacterial DNA-dependent RNA synthesis.[213] Rifampicin resistance is rooted in mutations that change the residues of the rifampicin binding site on RNA polymerase, which also leads to a lower affinity for rifampicin.[214] The possible to map resistant mutations to the *rpoB* gene, encoding RNA polymerase beta subunit.[215] Neri et al[216] reported different expressions of 23 proteins in two rifampicin-resistant and one susceptible meningococcus. Moreover, they report an increase in the proteins involved in the major metabolic pathways such as pyruvate catabolism and the tricarboxylic acid cycle; still, they showed a decrease in the proteins related to gene regulation in polypeptide folding. Rifampicin-resistant in a rifampicin resistant strain of *Brucella abortus* 2308 developed in vitro *was analyzed by* Sandalakis et al.[217] The resistant strain indicated the described mutation V154F, in the rpoB gene. Among 456 proteins found by MS/MS, the resistant strain had 39 differentially affected proteins that play a role in different metabolic pathways. Moreover, rifampicin resistance in *Brucella* is mostly effective in the excitation of many metabolic processes and possible use of the secretion mechanisms that exist at a more efficient level.[218] In general, these results indicate that rather than an outcome of changes in single proteins, resistance is the outcome of a complicated cellular processes network.

Proteomics Methods to Provide Mechanistic Insights in Bacterial Virulence

Growingly, proteomic techniques are attracting attention as key tools for studying bacterial pathogenesis.[134] Uses of these tools are finding of virulence factors and examining the response of both host and pathogen to infection. Provenzano et al[219] studied the metaproteome of microbial communities caused by endodontic infections featured with severe apical abscesses and asymptomatic apical periodontal lesions. They argued that many of the detected human proteins had a role in cellular processes and metabolism and immune defense. Wang et al[220] compared the proteome profile of the *S. enterica* subsp. enterica serovar

Typhimurium and *S. typhi*. These profiles are in charge of gastroenteritis and typhoid fever types. The authors first found a set of proteins with the serovar-specific expression as a novel biomarkers for finding clinical serotypes. They also reported that compared with *S. typhimurium*, the expression of flagella and chemotaxis proteins was lower in *S. typhi*. Mirrashidi et al[131] employed affinities purification-mass spectroscopy to find Inc-human interactions for 38/58 Incs that plays a role in intracellular life cycles of the host, including retromer components as sorting nexin. Observation of inc targets and overlapping of viral proteins indicates common pathogenic mechanisms among obligate intracellular microbes. In general, the findings mean that a better understanding of virulence factors and resistance mechanisms to antibiotics is achievable through realizing the functionalities of the involved proteins.

Conclusion

Using proteomic analysis gives us a valuable systematic approach to study the protein complement of bacterial pathogenesis. However, studies on using proteomic analysis to examine the interactions between bacterial pathogenesis and host are at early stages. That is, the new frontline of studies on pathogens is at the interface between the pathogen and host and examining the interaction of virulence proteins with cognate host entities, coordination of their actions, and finally subverting the host cell function as part of the disease process. However, we can use systems-level proteomic analyses to examine the intrinsically delicate balance of host-pathogen interactions. In addition, the host cells possess many defense strategies to defend against and kill invading pathogens. These key aspects of host-pathogen interactions are visible in proteomic differences. Research works on human infectious diseases have been extended notably thanks to proteomic approaches to pathogenic research. Proteomic tools are becoming promising ways for clinical studies and diagnosis. In another word, these proteomic studies have led to discoveries about different pathogenic infections by studying pathogenic factors, host anti-pathogen proteins, and protein complexes and profiling host and pathogen PTM sites during infection. The convergence of proteomics and omic technologies provides chances to have a clearer picture of the dynamics of diseases and find therapeutic targets. There is an immense potential for proteomic studies on PTMs to uncover mechanisms that mediate the progression, spread, and pathogenicity of infection.

Ethics Committee Approval

The present study was approved in National ethic committee with registration number IR.TBZMED.REC.1397.514.

Disclosure

The authors report no conflicts of interest in this work.

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