Review Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221-1691.385568

Impact Factor® 1.7

Metabolomics in drug discovery: Restoring antibiotic pipeline

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ABSTRACT

Metabolomics has emerged as a valuable tool in drug discovery and development, providing new insights into the mechanisms of action and toxicity of potential therapeutic agents. Metabolomics focuses on the comprehensive analysis of primary as well as secondary metabolites, within biological systems. Metabolomics provides a comprehensive understanding of the metabolic changes that occur within microbial pathogens when exposed to therapeutic agents, thus allowing for the identification of unique metabolic targets that can be exploited for therapeutic intervention. This approach can also uncover key metabolic pathways essential for survival, which can serve as potential targets for novel antibiotics. By analyzing the metabolites produced by diverse microbial communities, metabolomics can guide the discovery of previously unexplored sources of antibiotics. This review explores some examples that enable medicinal chemists to optimize drug structure, enhancing efficacy and minimizing toxicity *via* metabolomic approaches.

KEYWORDS: Metabolomics; Infectious diseases; Drug discovery; Antibiotics; Biomarkers

1. Introduction

Nowadays, the metabolomics approach is being utilized in the field of drug discovery and development, allowing researchers to identify new drug targets, monitor drug efficacy, and identify biomarkers for drug development[1,2]. By comparing the metabolite profiles of individuals with those of patients, specific metabolic signatures that are indicative of disease states can be identified. This comprehensive knowledge can be utilized to create new diagnostic tests for early disease detection and monitoring, as well as to identify potential drug targets. As the field of metabolomics progresses, it is likely to play an increasingly important role in identifying and quantifying the active metabolites within various biological samples such as urine, blood, and tissue samples[3].

Metabolomics also plays a substantial role in drug development by providing information regarding drug metabolism and toxicity. After assessing the metabolic fate of a drug, scientists can understand how the drug is processed and eliminated from the body which will help in determining the appropriate dosage regimens and predicting potential drug-drug interactions. This technology also helps to identify metabolites that are involved in disease processes and that may be targeted by drugs. For example, in antibiotic research, this approach has been used to identify metabolites that are involved in bacterial growth and that may be targeted by antibiotics[4]. By analyzing the metabolic changes that occur in response to a drug, researchers can determine whether the drug is having the desired effect and whether it is causing any adverse effects[5]. This

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How to cite this article: Azhar F, Busharat M, Chaudhary SRA, Waheed Z, Jamil MN. Metabolomics in drug discovery: Restoring antibiotic pipeline. Asian Pac J Trop Biomed 2023; 13(9): 378-383.

Article history: Received 5 July 2023; Revision 22 August 2023; Accepted 6 September 2023; Available online 21 September 2023

information can be used to refine drug dosing and to optimize drug regimens. A study reported that most of the metabolome researchers used liquid chromatography-mass spectrometry (LC-MS) (83%), followed by gas chromatography-mass spectrometry (30%), nuclear magnetic resonance (NMR) (26%), direct injection mass spectrometry (16%), image mass spectrometry (6%), matrix-assisted laser description/ionization mass spectrometry (4%), and capillary electrophoresis-mass spectrometry (3%) for the drug discovery and development[6,7].

In this overview, we examine a few examples where metabolomics approaches are employed to revive the antibiotic pipeline. These approaches empower medicinal chemists to refine drug structure, thereby improving effectiveness and reducing toxicity.

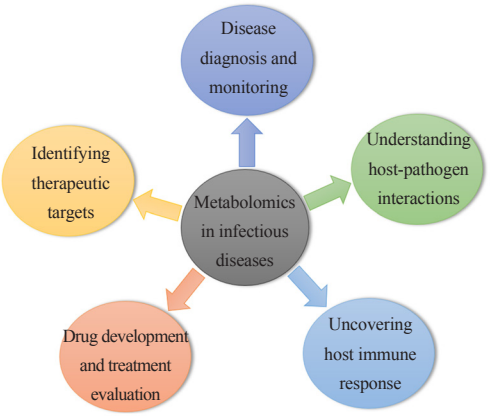

Figure 1. Role of metabolomics in infectious diseases.

2. Metabolomics in infectious disease

The role of metabolomics in infectious diseases has become increasingly important over the years as it offers unique insights into the pathophysiology of infections and can provide new avenues for the development of novel therapies[8]. Metabolomics can be applied to infectious diseases in various ways (Figure 1). Firstly, it can be used for the discovery of new biomarkers that could aid in the diagnosis of infections[9,10]. Metabolites such as amino acids, lipids, and organic acids can be used to identify the presence of specific infectious agents or to distinguish between different types of infections[11]. Additionally, metabolomics can be used to monitor the progression of infections and assess the effectiveness of treatments.

Metabolomics can also help in the identification of new drug targets for infectious diseases. For example, Pacchiarotta and his colleagues used LC-MS technology to study the metabolomics of urinary tract infections (UTIs)[12]. The prospective observational cohort study was carried out where the authors enrolled patients who were diagnosed with confirmed *Escherichia coli* UTIs. The samples of control and UTI patients were collected at the baseline and subsequently after receiving antibiotics. The authors not only compared the symptoms of UTI patients with control but also noticed how the metabolic phenotype of the patients reverts after treatment. During this study, they discovered *O*-glycosylated fragments of human fibrinogen alpha-1 chain that exhibited a strong correlation with UTI symptoms.

Understanding the mechanism of action of existing antibiotics using this approach can result in the development of new and more effective therapies. Moreover, metabolomics can reveal changes in host metabolism that occur in response to infection and identify host factors that are important for the development of infections, which can aid in the development of new preventive strategies.

3. Metabolomics in antibiotic discovery

One of the greatest accomplishments in modern medicine is the discovery and development of antibiotics for the prevention and treatment of infectious diseases. However, the bacterial infections caused by multi-drug resistant pathogens are increasing worldwide which poses a threat to public health[13]. The discovery of novel antibiotics has lasted since the 1960s (Figure 2)[14,15]. As most of the antibiotics such as tetracyclines or aminoglycosides are extracted from actinomycetes *via* bioactivity-guided antibiotic isolation approaches. Natural products are preselected candidates in terms of lead compounds in antibiotic research as they are derived from respective pathogens or plants to protect themselves against competitors or predators in their ecological niches. Natural product-based antibiotics have faced more challenges, such as discovery of lead compounds from well-known bacterial stains, as well as those that feature intense mass-spectrometry or ultra-violet spectroscopy signals[16].

Antibiotic discovery is an ongoing challenge, as the development of new antibiotics is necessary to combat the growing problem of antibiotic resistance. Metabolomics has emerged as a promising tool for antibiotic discovery, as it allows researchers to study the metabolic pathways that are involved in bacterial survival and growth, and to identify potential targets for new antibiotics. One application of metabolomics in antibiotic discovery is to identify metabolites that are essential for bacterial growth and survival. By targeting these metabolites, researchers can develop new antibiotics that selectively target bacterial cells, while leaving the host organism unharmed. Another role of metabolomics in antibiotic discovery is to identify metabolites that are produced by bacteria in response to antibiotic exposure. These metabolites, known as "secondary metabolites", may have antibiotic activity themselves, or may provide clues to the metabolic pathways that are involved in antibiotic resistance.

Lu *et al*. used integrative strategies such as taxonomical information, bioactivity, and metabolomic tools [principal component analysis (PCA), latent structures discriminant analysis, and molecular networking] for dereplication that allowed them to prioritize two strains of *Streptomyces* (H37, M22) with the potential to develop novel antibiotics[17]. Moreover, another study applied LC/MS-PCA to develop new antibiotics from marine-derived *Streptomyces* species[18]. Discovery of novel antibiotics usually includes a risk assessment of spread and emergence of potential resistance. Another approach for reusing the existing antibiotics is to modify their chemical structure which integrates two or more pharmacophores, for example, kanglemycin-fluoroquinolone, rifamycin-nitroimidazole conjugate, chimeric streptogramin-tyrocidine rifamycin-quinolone hybrid drug and other hybrid constructs having various antibiotic pharmacophores[19-23]. Moreover, high-throughput screening and genetic engineering can also help to improve existing antibiotic peptides[7].

4. Metabolomics in antibiotic mode of action and physiological response markers

Metabolomics provides potentially rapid and cost-effective means of identifying antibiotic mode of action and physiological response markers[24]. These approaches can help in the identification of the metabolic changes that occur in bacteria following exposure to antibiotics, providing a comprehensive understanding of the mode of action of antibiotics and the physiological response of bacteria to treatment. Moreover, metabolomics can be used to identify physiological response markers for antibiotic treatment[25]. The workflow for biomarker discovery and validation using a metabolic approach is illustrated in Figure 3. The metabolites can serve as biomarkers to evaluate the efficacy of antibiotics and the development of antibiotic resistance. Metabolomics can also be used to identify the mechanisms of action of antibiotics[4]. Its ability to provide detailed information about metabolic pathways and mechanisms of action can aid in the development of new antibiotics and the monitoring of antibiotic treatment. As the field of metabolomics continues to advance, it is likely to play an increasingly important role in the fight against bacterial infections.

Metabolomics techniques utilizing NMR methods provide a reliable and precise screening platform for classifying novel antibiotics based on their mechanisms of action. This approach proves to be a valuable alternative to High Throughput Screening evaluation due to its efficiency, accuracy, and reproducibility. A recent study has successfully reported the identification of metabolomic changes

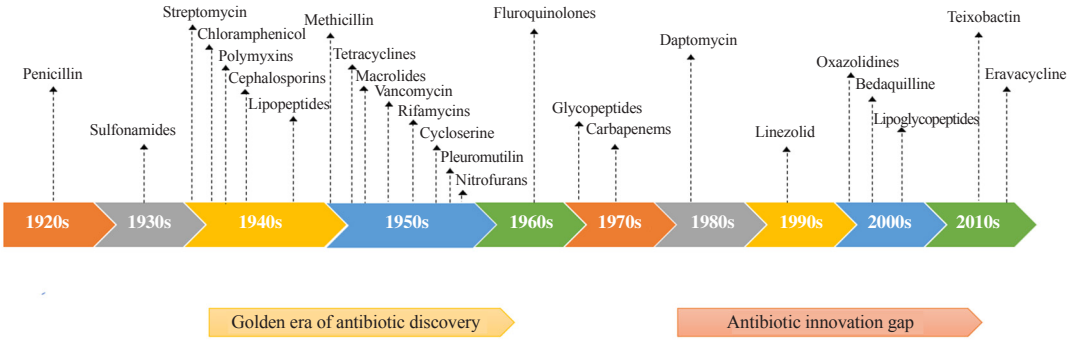

Figure 2. Timeline of the discovery of antibiotics.

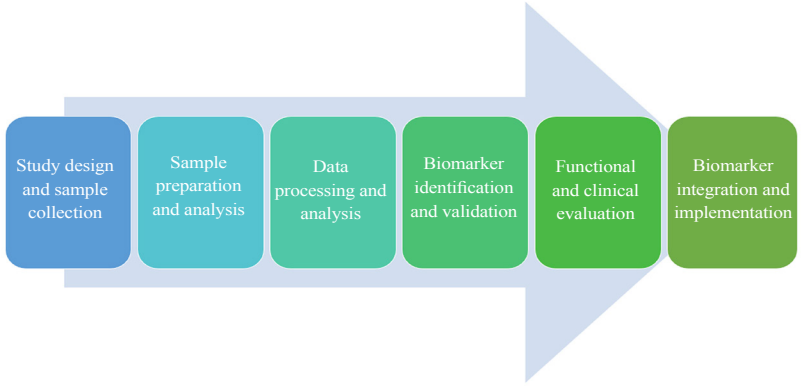

Figure 3. Workflow for biomarker identification and validation.

associated with antibacterial activity, including cell wall damage and inhibition of mycolic acid production, using a metabolome model of *Mycobacterium smegmatis*[26,27]. Moreover, metabolomics has been utilized to forecast the mode of action of various antimicrobial drugs in different microbial models. For instance, in the case of *Tinospora capillipes* extract, the antifungal activity and mechanism of action were determined using liquid chromatography-electrospray ionization-tandem mass spectroscopy metabolomic analysis. This approach enabled a qualitative examination of the exometabolome, which encompasses the metabolites secreted into the fermentation media[27].

Antibiotic mode of action can be determined by various strategies such as pathway analysis, classification strategy, or incorporation of metabolomics, genomics, and transcriptomics. However, both the integrative approach and comparative metabolomics help in identifying the bacterial mechanism of resistance[28]. J-resolved, total correlation spectroscopy, correlation spectroscopy, heteronuclear multiple-bond correlation, and heteronuclear single quantum coherence are the most used techniques in NMR metabolomics. The parameters of NMR including, spectral data preprocessing, NMR measurements, and multivariate data analysis use a protocol that was originally designed for the analysis of plant metabolites[29].

5. Metabolomics in predicting side effects

Antibiotics can have a wide range of side effects, including disruptions to the microbiome, host cell destruction, and liver toxicity[30,31]. These adverse events can be difficult to identify and may not manifest immediately which makes them challenging to monitor. Metabolomics can also help to identify metabolic changes that occur in response to antibiotic treatment and can provide insights into the mechanisms by which antibiotics cause side effects[8]. Scientists can identify metabolites that are altered in response to antibiotic treatment by examining the metabolic profiles of host cells and the microbiome.

A study examined the potential nephrotoxic side effects of gentamicin using a proton nuclear magnetic resonance (^{1}H NMR) and revealed that the altered metabolites such as 3-hydroxybutyrate, glucose, citrate, alanine, and glycine might serve as specific and sensitive biomarkers for gentamicin-induced nephrotoxicity[32]. Similarly, in another study, researchers explored the hepatotoxic effects of erythromycin in albino Wistar rats and analyzed the changes in liver metabolites using NMR-based metabolomics. This approach provided valuable insights into the metabolic alterations associated with erythromycin-induced liver toxicity, aiding in understanding the adverse effects of these antibiotics on liver function[33].

6. Limitations and challenges

Although metabolomics is an effective technology for drug development, several limitations and challenges must be discussed. The complexity of the data generated is one of metabolomics' main drawbacks. A variety of biological and technical factors such as the preparation of samples, data processing, and data analysis might have an impact on the interpretation of metabolomic data and make it more complex to identify the specific metabolic pathways and targets for antibiotic discovery[34]. Moreover, the lack of standardization in metabolomic approaches is another challenge[35]. The reproducibility and reliability of the metabolomic data can be affected by various metabolomic approaches used by different laboratories. The comparison of data between studies might be hampered by the lack of standardization, making it more challenging to draw desired outcomes. Additionally, the availability of large samples required for the analysis of metabolites can be sometimes difficult to obtain, especially for rare illnesses or illnesses that affect vulnerable populations.

Multiple confounding factors, such as lifestyle modification and environmental exposures, can affect the metabolic profile of people and can make it difficult to identify specific metabolic pathways and targets for drug discovery[36]. Therefore, to address limitations and challenges, the standardization of methodologies, the availability of suitable samples, and the development of robust analytical as well as statistical tools will be required. Metabolomics is likely to play an increasingly important role in drug discovery and personalized medicine when these challenges are resolved.

7. Future perspectives

However, metabolomics technology is the most effective method for diagnosing infectious diseases and formulating new antibiotics. While this technology can be expensive and analytically challenging, therefore, separation methods should be modified to improve the portability and reliability of metabolomic technologies in the search and discovery of promising compounds. To address this challenge, separation techniques in the form of microfluidic systems, such as microchip-CE, should have been implemented[37]. This miniaturized version of liquid chromatography involves the detection of primary and secondary metabolites, which is directed toward various purposes such as monitoring, clinical diagnostics, and toxicity screening.

Another interesting future trend involves the utilization of NMR and LC-MS in nanotoxicity studies that aim to assess the adverse events of nanoparticles and reveal the mechanism of action of both nanomaterials as well as nano-formulations[38,39]. Furthermore,

metabolomics proves to be a fascinating approach for improving the efficacy of chemically engineered extracts[40,41]. This innovative method represents a potential source for identifying new drug candidates with a significant impact by utilizing the chemical diversity found in both known and unknown natural scaffolds. Ultimately, it could herald a new era in the synthesis of laboratory-generated secondary metabolites destined for applications in industry, medicine, and agriculture, thereby opening new prospects for future drug development.

8. Conclusion

Metabolomics has the potential to restore the antibiotic pipeline by facilitating the discovery of novel antimicrobial compounds and optimizing their efficacy. By providing a comprehensive understanding of the metabolic changes induced by antibiotics, metabolomics enables the identification of new drug targets and the development of more effective therapeutic strategies. With its ability to screen natural products and assess drug metabolism and toxicity, metabolomics represents a valuable tool in the fight against antibiotic resistance and the development of urgently needed antibiotics.

Conflict of interest statement

The authors declare no conflict of interest.

Funding

The authors received no extramural funding for the study.

Authors' contributions

FA and MB designed the manuscript, SRAC and ZW were involved in data collection required for figures. FA and MNJ edited the final version of manuscript.

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