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Study on Antibiotics Residues and Antibiotic Resistance Detection Techniques by Raman Spectroscopy

Shuyi Guo

Faculty of Chemistry, Northeast Normal University, Changchun, China
guoshuyi@nenu.edu.cn

Abstract: In recent years, as people's standard of living rises, the need for meat and dairy products has increased greatly. From the perspective of antibiotic residues, it may be not a promising trend. Excess antibiotics can be accumulated through the food chain and finally become a threat to human health and the environment. Thus the detection and supervision of antibiotics residues play an important role in sustainable development. Also, the problem of antibiotic resistance has been increasingly serious nowadays. Before antibiotics were invented, there were many outbreaks caused by bacteria such as the Black Death, the Plague, and Anthrax. During that time, plenty of people died of bacterial infection, which has become an indelible wound in the history of mankind. Similarly, if pathogenic bacteria develop extensive antibiotic resistance, human health will be at great risk. Finding the right approaches to detect pathogen resistance is crucial. However, existing methods such as liquid chromatography and mass spectrometry are unable to meet the rapidly growing demand for swift and efficient detection. Raman spectroscopy, a non-destructive and non-contact method, can provide rapid information on the chemical structure, physical form, crystallinity and intermolecular interactions of the sample under test. Owing to these specificities, this method is valuable for purely qualitative analyses, highly quantitative analyses and molecular structure determination like antibiotic residues and antibiotic resistance detection. Together with artificial intelligence(AI) like machine learning, Raman spectroscopy will be used more widely.

Keywords: Raman spectroscopy, antibiotic abuse, antibiotics residues detection, antibiotic resistance detection, machine learning

1. Introduction

The misuse of antimicrobial drugs in livestock raising and even in the cultivation of fruits and vegetables has resulted in the residues of antimicrobial drugs on food. Behind this phenomenon, the most significant negative impact is an increase in bacterial resistance, which not only affects food safety but also may have long-term effects on consumers' health. Some studies show that antibiotic residues can cause health problems such as allergies, hepatitis, gastrointestinal disorders, carcinogenicity and teratogenicity [1]. What's worse, if the individuals are exposed to low doses of antibiotics for a long time without knowing it, the levels of antibiotic residues in their bodies will be increased, which can hugely threaten human health.

Besides the antibiotics residues, the danger of antibiotic resistant cannot be ignored, too. WHO predicts that drug-resistant diseases could lead to 10 million deaths per year by 2050 if nothing is

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done [1]. In clinical practice, not only does the misuse of antimicrobial drugs fail to curb the progression of the disease, but can further lead to the spread of bacterial resistance. Thus, finding an appropriate way for rapid detection of causative organisms and their resistance is the key to rapid diagnosis of etiology and accurate drug administration. However, the current mainstream methods can not meet the demand for rapid detection. Because of this reason, the development of assays for the detection of bacterial resistance has received a great deal of attention.

In the past 10 years, research on the application of Raman spectroscopy in clinical diagnosis has gained more and more attention, making it possible for rapid detection. Raman spectroscopy and its relevant methods, using vibrational spectroscopy, have the advantage of rapid detection and instrument miniaturisation, which provide great developmental space for realising on-site, rapid and accurate identification of pathogenic bacteria and their drug resistance. In this paper, it will briefly introduce the principles of Raman spectroscopy and mainly focus on the application of Raman spectroscopy techniques for antibiotic residues and antibiotic resistance detection, with the reference of the progress of Raman spectroscopy in this area recently.

Above all, by summarizing and organizing the existing literature about Raman spectroscopy, the article will provide new ideas to solve the current problem of antibiotic-related detection, generally faced by today's society in many aspects. Also, the use of chemical assays in biology is one of the trends in the research of life sciences today. With the help of multi-dimensional detection technologies, the detection of antibiotics residues and antibiotic resistance can be improved in terms of accuracy, convenience and timeliness.

2. Raman spectroscopy

Raman spectroscopy is a molecular vibrational light based on the Raman scattering effect and its identification of molecular vibrational state is achieved by inelastic scattering spectroscopy. In biological applications, it is highly specific for observing changes in biomolecules through specific spectral pattern recognition [2]. Raman spectroscopy can detect the characteristic fingerprint spectrum unique to each substance. Undisturbed by water, this method suits well to the study of biomedical systems [3]. However, due to its poor sensitivity, Raman spectroscopy typically requires analytes to be present in molar to millimolar concentrations for effective detection.

To address this limitation, a more sensitive technique known as Surface-Enhanced Raman Spectroscopy (SERS) has been developed. It can generate surface plasmonic excitations by photoexcitation of special substrates like metal nanoparticles (usually Au, Ag, Cu, etc.), through which Raman scattering signal of adsorbed molecules is enhanced by 106 ~1012 times [4]. Among various materials, silver nanoparticles (AgNPs) are widely used due to their superior performance in enhancing Raman signals, particularly in the detection of pathogenic bacteria. Given its high sensitivity, high specificity and short detection time, there has been a great deal of interest in SERS to be used in sensitive detection and physiological activity analysis of bacterial microorganisms.

SERS-based assays have been reported in the areas of food safety, environmental testing and clinical diagnostics related to microbiological testing. For instance, Girmatsion group first used SERS technology to perform detailed spectral analysis of various antibiotics such as penicillins and fluoroquinolones. By designing suitable SERS active substrates, the antibiotic molecules formed a stable adsorption layer on the surface of the substrate, which in turn generated strong Raman scattering signals. Besides, the team has successfully obtained the unique SERS spectral fingerprints by collecting and analyzing these signals [5].

However, several defects still remain in SERS detection technologies, such as the poor reproducibility in detecting substrate materials and the complexity of the detection process, limiting their wide application in food safety monitoring and clinical application and remaining to be upgraded [6]. Due to the lack of standardized Raman spectral databases for the identification of

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clinical pathogens, the attribution of Raman shift peaks to pathogens is unclear. Meanwhile, although label-free SERS techniques have already provided the characteristic Raman spectral information of pathogens, they have rarely been reported for the analysis of drug resistance in pathogenic bacteria. Thus, there is still huge space for the improvement of Raman spectroscopy in the area of antibiotic testing.

3. The problem of antibiotics

3.1. Antibiotics residues

3.1.1. Antibiotic abuse

Antibiotics are general terms for chemical substances that can specifically inhibit the vital activity of certain organisms at low concentrations, interfering with the normal developmental functions of other cells. There are mainly eight kinds of antibiotic used in the food of animal origin: Fluoroquinolones(FQs), β-lactams(BLAs), Macrolides(MA), Tetracyclines(TCs), Nitrofurans(NFs), Sulfonamides(SAs), Amphenicols and Aminoglycosides(AGs) [7]. While they each exhibit different effects in various contexts, they share similar antimicrobial mechanisms by interfering with bacterial physiological processes, such as cell wall synthesis, protein synthesis, and DNA replication.

Although their mechanisms of action are selective, primarily targeting bacterial cells and being less toxic to host cells, antibiotics can still pose risks to both the environment and human health. If they are partially absorbed into the animal's body, the unabsorbed portion is excreted as either prototypes or metabolites into the environment, potentially threatening both ecological systems and human health. For example, quinolone antibiotics are widely used for the prevention and treatment of bacterial infections and also work as feed additives. Their misuse can lead to excessive antibiotic residues in foods such as poultry meat, milk, honey and other agricultural products [8, 5]. Whether antibiotics remain in the body of livestock or are discharged into the environment, they will eventually enter the human body directly or indirectly through the food chain, greatly endangering human health like producing allergic reaction, altering the human microflora and even resulting in antibiotic resistance in human.

3.1.2. Antibiotic residues detection

In response to the serious problem of antibiotic abuse, many countries have regulated the use of a range of antibiotics [1]. With the purpose of protecting the public health, reducing antibiotic residues and antibiotic resistance and promoting sustainable agricultural development, these policies require timely and on-site quantitative testing for antibiotic residues in the products to make sure that the indicators are under certain levels.

In recent years, with the development of nanotechnology, researchers have established a series of rapid and sensitive detection methods based on SERS technology, especially in the field of common food contaminants, such as foodborne pathogens, drug residues, food additives and illegal chemical additives. For example, Hassan group designed a SERS sensor based on silver nanoparticles combined with solid-phase extraction for the rapid quantitative determination of methomyl, acetamiprid and 2,4-D residues in green tea. And Dhakal group developed a simple surface enhanced Raman spectroscopy method, which directly employs silver sol-gel nanoparticles for in situ detection of tetracycline residues in milk and water with detection limits as low as 0.01 mg/L [8].

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3.2. Antibiotics resistance

With the abuse of antibiotics, multi-drug resistant bacteria are emerging at an unprecedented rate. Because of this, the effectiveness of antibiotic treatment is on the wane. It is predicted that antibiotic-resistant bacterial infections will overtake cancer as the leading cause of death [9]. However, the traditional methods like liquid chromatography and mass spectrometry are so time-consuming that doctors often need to use broad-spectrum antibiotics first, which tends to lead to an increase in antibiotic resistance. Thus, a rapid, automated and reliable antimicrobial susceptibility testing (AST) is urgently needed especially in the clinic to guide the precise use of antibiotics. Recently, Raman spectroscopy has gained an increasing attention in clinical diagnosis, which can detect changes in bacterial metabolism rapidly and non-destructively. For thess strengths, it is expected to solve the problem by using Raman spectroscopy to monitor deuterium absorption to achieve rapid and reliable AST [10].

Kai group developed a rapid AST method combined with single-cell Raman spectroscopy and heavy water labelling (Raman-D2O), which is directly applicable to clinical urine samples. And, Yi 's team established a rapid, simple and direct Raman spectroscopy-assisted drug sensitivity test, through which the AST value can be read out within 3 h for urine samples and 21 h for blood samples, greatly improving the efficiency of clinical testing [10]. The two examples reflect Raman spectroscopy's superior to standard culture-based methods in terms of time-consumption, meaning that it may become an important tool for assessing antibiotic susceptibility.

Rapid detection of the causative organisms and their resistance facilitates the prevention and rapid diagnosis of drug-resistant bacterial infections, as well as scientifically guided "precision medication" in the treatment phase. Compared with sensitive bacteria, antibiotic-resistant bacteria have differences in molecular structure. By collecting Raman spectral information from both samples and combining them with multivariate statistical analysis, it is possible to quickly distinguish between resistant and sensitive bacteria. Popp's group, which has achieved fruitful research results in this field, has presented the basic concept of drug diagnosis using Raman spectroscopy. The application of Raman spectroscopy can play a good role in the detection and the identification of bacteria in body fluids of patients with urinary tract infections [11], as well as the rapid spectroscopic qualitative, quantitative detection and the analysis of antibiotic-resistant or susceptible bacteria [1].

In addition, when adding specific substances to the culture of bacteria, the bacterial metabolic process will be affected, and then Raman spectroscopy can detect the changes in bacterial growth for pharmacological sensitivity tests. For example, if a medium containing D2O is used in the cultivation of the bacteria, deuterium is involved in the process of lipid synthesis with the bacteria growing. The C-H bond is replaced and the rate of replacement correlates linearly with the cellular metabolic activity. Raman spectroscopy can detect this process quantitatively. Therefore, the change in the rate of C-D bond substitution in Raman spectra after treatment with different antibiotics can be compared to characterise the strength of the metabolic activity of the bacteria. This rapid drug sensitivity test can be achieved in less than 1h without incubation [10].

However, Raman spectroscopy is susceptible to interference from other components in the sample, affecting the specificity of the test. Moreover, the process requires a large instrument such as a Raman spectrometer, which is costly and the application is narrow in scope. The use of Raman spectroscopy alone for the detection of pathogen resistance still has limitations, and its use in conjunction with other techniques, such as the isotope labelling mentioned above, can be better.

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4. Discussion

In order to improve the speed of spectral analysis and recognition accuracy, there is a growing trend to combine SERS detection technology with machine learning, which can effectively improve the recognition ability, differentiation ability and prediction ability of multiple bacterial pathogens [12]. Recently, Ciloglu et al. applied machine learning in conjunction with SERS technology for the rapid detection of mucin-resistant Klebsiella pneumoniae. By using autoencoder and PCA to extract nonlinear and linear features in spectral data respectively, the effective feature extraction was achieved, revealing the spectral differences between resistant and susceptible strains. The analytical algorithm demonstrated excellent classification performance with an accuracy of 94.2% [13]. This highlights the potential of machine learning to address the challenges posed by the high volume and complexity of SERS spectral analyses in antimicrobial susceptibility testing (AST) experiments.

In the field of microbiological Raman spectroscopy, the one-dimensional convolutional neural network is the most commonly used model. Yu's team extracted four species of marine bacteria from sea intestines and used a simple convolutional neural network model with only two convolutional layers and one fully connected layer to classify their SERS Raman spectra, achieving an accuracy of 94% [14]. In addition to the one-dimensional convolutional neural network algorithm, the ReusNET method has also attracted much attention in the classification of bacterial Raman spectra. To identify 30 common pathogens, Zhou's group used a Rees Neut model with a depth of 26 to classify Raman spectra with a low signal-to-noise ratio (SNR). The accuracy of the identification of 30 pathogen isolates was 92%. Similarly, in the task of the Antibiotic Experimental Treatment Group (AETG), an accuracy of 97% was achieved [15].

Although machine learning has significantly improved the level of research in pathogen Raman spectroscopy, its application in Raman spectroscopy still faces many challenges. Training and data preparation are the most critical issues. There are no large general-purpose open-source Raman spectral datasets that can be used to pre-train deep learning models for transfer learning, and small Raman datasets may lead to poor algorithmic performance. Therefore, in order to solve these problems, joint efforts from all over the world should be made to help Raman spectroscopy be more combined with AI such as one-dimensional convolutional neural networks and ReusNET, aiming to set up a large database worldwide and then find the most appropriate way to improve its practical ability and finally benefit humanity and the environment.

5. Conclusion

Behind the rich diet, there exists the problem of excessive antibiotic residues, which can accumulate in the environment and the human body and eventually cause serious harm. In addition, due to the misuse and overuse of antibiotics, the impact of bacterial resistance on healthcare has become a global concern. The outstanding superiority of Raman spectroscopy in rapid and accurate detection has already been promising in the research of pathogenic bacteria detection. This paper take antibiotic residues and antibiotic resistance as examples to illustrate the practical application of Raman spectroscopy and its unique advantages, hoping that this method can be further improved and then can be widely used to protect the health.

The utilization of Raman spectroscopy can be of major significance in detecting antibiotic residues quickly, efficiently and accurately. Moreover, by collecting highly specific molecular fingerprints of pathogens to distinguish different strains, the action characteristics of antibiotics can be determined to identify drug-resistant strains. Additionally, AI technology can enhance the comprehensive analysis and processing ability of Raman signals acquired by the equipment, though this requires the establishment and refinement of Raman spectral libraries and the mutual comparison and verification of the research results of different subject groups.

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This article still has some shortcomings. Due to the lack of experimental conditions such as sufficient samples for antibiotic residues or antibiotic resistance, there is no empirical research behind the paper. And merely reviewing some new articles that published in recent years, the scope and depth of the literature search are not sufficient. However, what the article tries to convey is that the use of chemical methods such as Raman spectroscopy cannot be ignored in the current development of biology. Improvements in detection methods may greatly contribute to the advancement of biology, thus helping to solve some of the difficulties and benefiting human health. The cross-fertilisation of chemistry and biology will inject fresh blood into the field of life sciences, leading to more comprehensive and in-depth scientific research in the future.

References

[1] *Zhang G., Chen B.R., Pan L.L., Wang H., Yang B. & Bu Q.P. (2024). Research status and rapid detection methods of antibiotic residues in agricultural products. Chinese Journal of Applied Ecology, 35(05), 1408-1418. doi:10.13287/j.1001-9332.202405.031.*

[2] *Zhang P., Chu D.C., Chen K.R., Liu Y.L., Luo R.H., Liu Y. & Zheng D.W. (2020). Recent Advances in Rapid Detection of Antibiotic Resistant Bacteria via Raman Spectroscopic Technology. Journal of Beijing University of Technology, 46(12), 1417-1424. doi:10.11936/bjutxb2019030010.*

[3] *Fu,S.J. (2020). Evaluation of Bacterial Susceptibility to Antibiotics Based on Surface Enhanced Raman Spect roscopy. Master thesis. Changchun University of Science and Technology, Jilin. doi:10.26977/d.cnki.gccgc.20 20.000189.*

[4] *Chen X.P. (2019). Surface-enhanced Raman spectroscopy for genus identification and drug resistance analys is of pathogenic bacteria. Doctoral thesis. Army Medical University,Chongqing. doi:10.27001/d.cnki.gtjyu.201 9.000305.*

[5] *Zhang, Z.X, Wang, J.W., Wang, C.X. & Qin, M. (2025). SERS Determination of Enrofloxacin Antibiotic residues in Milk. Shandong Chemical Industry, 54(02), 169-172+179. doi:10.19319/j.cnki.issn.1008-021x.2025.02.039.*

[6] *Huang Z.X., Li Z.Y., Zhu Y.T., Zhang T., Wen Y.Q. & Wang H.X. (2024). Research Advances in SERST ech nology for Assessing Bacterial Drug Resistance. Chemistry, 87(05), 550-558+549. doi:10.14159/j.cnki.0441-3 776.2024.05.005.*

[7] *Fu, H.Y., Lu, H.H., Long, W. J. & She, Y. B. (2023). Progress in the detection methods of antibiotic residues in animal derived food and research. Journal of Light Industry, 38(06),37-45. DOI: 10.12187/2023.06.005.*

[8] *Ren L.Y. (2024). Theoretical and experimental study on surface-enhanced Raman spectroscopy of quinolone antibiotics. Master thesis. Mudanjiang Normal University, Heilongjiang. doi:10.27757/d.cnki.gmdjs.2024.000338.*

[9] *Tao Y, Wang Y, Huang S,Zhu P, Huang WE, Ling J, Xu J. Metabolic-activity-based assessment of antimicrobial effects by d(2)o-labeled single-cell raman microspectroscopy. Anal Chem, 2017, 89:4108-4115.*

[10] *Zhang, Y.Y. (2024). Characterisation of Clostridium perfringens resistance to macrolides and determination of Raman spectra. Master's thesis. Huazhong Agricultural University, Hubei. doi:10.27158/d.cnki.ghznu.2024. 001592.*

[11] *Tannert, A., Grohs, R., Popp, J., & Neugebauer, U. (2019). Phenotypic antibiotic susceptibility testing of pathogenic bacteria using photonic readout methods: recent achievements and impact. Applied Microbiology and Biotechnology, 103, 549-566.*

[12] *Li Y., Qi Y. & Li H. (2022). .Advances of Raman Spectroscopy in Diagnosis of Infectious Diseases. Chinese Journal of Analytical Chemistry, 50(03), 317-326. doi:10.19756/j.issn.0253-3820.210810.*

[13] *Ciloglu, F.U., Hora, M., Gundogdu, A., Kahraman, M., Tokmakci, M., Aydin, O. (2022, Aug 15). SERS-based sensor with a machine learning based effective feature extraction technique for fast detection of colistin-resistant Klebsiella pneumoniae. Anal Chim Acta. 1221:340094. DOI:10.1016/j.aca.2022.340094. Epub 2022 Jun 15. PMID: 35934394.*

[14] *Ho, C. S., Jean, N., Hogan, C. A., Blackmon, L., Jeffrey, S. S., Holodniy, M., Banaei, N., Saleh, A. A. E., Ermon, S. & Dionne, J. (2019). Rapid identification of pathogenic bacteria using Raman spectroscopy and deep learning. Nature communications, 10(1), 4927.*

[15] *Zhou B. (2023). Mechanism and Application of Raman Optical Tweezers for Rapid Detection of Pathogenic Bacteria. Doctoral thesis. Beijing University of Posts and Telecommunications, Beijing. doi:10.26969/d.cnki.g bydu.2023.000387.*