Mini Review
doi.org/10.1002/ansa.202200066
Chemistry Europe
European Chemical Societies Publishing

Received: 26 December 2022
Revised: 3 March 2023
Accepted: 10 March 2023

Recent advances of Raman spectroscopy for the analysis of bacteria

Linsey Rodriguez[1] | **Zhiyun Zhang**[2] | **Danhui Wang**[1]

[1]Department of Nutrition and Food Sciences, Texas Woman's University, Denton, Texas, USA

[2]Research and Development, Daisy Brand, Garland, Texas, USA

Correspondence
Danhui Wang, Department of Nutrition and Food Sciences, Texas Woman's University, Denton, TX 76204, USA.
Email: dwang4@twu.edu

Funding information
Research Enhancement Program at Texas Woman's University

Abstract

Rapid and sensitive bacteria detection and identification are becoming increasingly important for a wide range of areas including the control of food safety, the prevention of infectious diseases, and environmental monitoring. Raman spectroscopy is an emerging technology which provides comprehensive information for the analysis of bacteria in a short time and with high sensitivity. Raman spectroscopy offers many advantages including relatively simple operation, non-destructive analysis, and information on molecular differences between bacteria species and strains. A variety of biochemical properties can be measured in a single spectrum. This short review covers the recent advancements and applications of Raman spectroscopy for bacteria analysis with specific focuses on bacteria detection, bacteria identification and discrimination, as well as bacteria antibiotic susceptibility testing in 2022. The development of novel substrates, the combination with other techniques, and the utilization of advanced data processing tools for the improvement of Raman spectroscopy and future directions are discussed.

Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional; 4-MPBA, 4-Mercaptophenylboronic Acid; *Aa*, *Aggregatibacter actinomycetemcomitans*; AAS-NPs, Au-Ag-stuffed nanopancakes; AGNES, Agglomerative nesting; AgNPs, Silver nanoparticles; AuNCs, Gold nanocomposites; AuNPs, Gold nanoparticles; AuNS, Gold nanoshell; Au@Ag, NPs Au@Ag nanoparticles; Au@MNP, Gold magnetic nanoparticles; WGA, Wheat germ agglutinin; Au NNPs, Gold nano-bridged nanogap nanoparticels; ConA-Fe_3O_4@SiO_2 NPs, Concanavalin A-conjugated Fe_3O_4 modified SiO_2 nanoparticles; *B. amyloliquefaciens*, *Bacillus amyloliquefaciens*; *B. cereus*, *Bacillus cereus*; *B. licheniformis*, *Bacillus licheniformis*; *B. subtilis*, *Bacillus subtilis*; BCNC, Bacterial cellulose nanocrystal; *C. acnes*, *Cutibacterium acnes*; *C. difficile*, *Clostridium difficile*; *C. jejuni*, *Campylobacter jejuni*; *C. indologenes*, *Chryseobacterium indologenes*; *C. parvum*, *Cryptosporidium parvum*; *C. perfringens*, *Clostridium perfringens*; *C. santamariae*, *Candida santamariae*; CNN, Convolutional neural network; Con A, Concanavalin A; DFA, Discriminant function analysis; DualWKNet, Dual-branch wide-kernel network; *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; *E. piscicida*, *Edwardsiella piscicida*; Fe_3O_4, Iron-oxide; *Fn*, *Fusobacterium nucleatum*; GAN-SVM, Generative adversarial network and multiclass support vector machine; GO, Graphene oxide; HCA, Hierarchical cluster analysis; KNN, k-nearest neighbor; *L. mesenteroides*, *Leuconostoc mesenteroides*; *L. monocytogenes*, *Listeria monocytogenes*; LDA, Linear discriminant analysis; LFAs, Lateral flow assays; LOD, Limit of detection; LS-SVM, Least-squares support vector machine; MIC, Minimum inhibitory concentration; MPN@Con A, Concanvilin A functionalized magnetic nanoparticle; NC-NB, nitrogen doped carbon nanoballoon; NMPs, Nanomica platelets; NSP, Nanoscale silicate platelet; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. pabuli*, *Paenibacillus pabuli*; *P. plecoglossicida*, *Pseudomonas plecoglossicida*; PCA, Principal component analysis; PC-LDA, Principal components-linear discriminant anlaysis; PDMS, Polydimethylsiloxane; *Pg*, *Porphyromonas gingivalis*; PLS-DA, Partial least square discriminant analysis; *S. aureus*, *Staphylococcus aureus*; *S. cohnii*, *Staphylococcus cohnii*; *S.* Derby, *Salmonella* Derby; *S. enterica*, *Salmonella enterica*; *S.* Enteritidis, *Salmonella* Enteritidis; *S. epidermidis*, *Staphylococcus epidermidis*; *S. flexneri*, *Shigella flexneri*; *S. hominis*, *Staphylococcus hominis*; *S. paratyphoid*, *Salmonella paratyphoid*; *S.* Typhimurium, *Salmonella* Typhimurium; SASP, Self-assembled solid-phase; SERS, Surface-enhanced Raman scattering; SVM, Support vector machine; TBDP, Tert-Butyl 2,4-dioxopiperidine-1-carboxylate; TCP, Teicoplanin; TPP, Tobacco packaging paper; *V. anguilarum*, *Vibrio anguilarum*; *V. harvevi*, *Vibrio harvevi*; *V. parahemolyticus*, *Vibrio parahemolyticus*.

Mini Review
doi.org/10.1002/ansa.202200066

1 | INTRODUCTION

Rapid and sensitive detection and identification of bacteria are becoming increasingly important with the continual need to monitor the presence and activity of bacteria for the control of food safety, the prevention of infectious disease, and environmental monitoring.[1,2] The most commonly used methods for bacteria detection and identification are culture-based methods which are reliable and well-established.[3] However, culture-based methods are time-consuming, labour-intensive, and unable to meet the rapid requirement of bacteria detection. Improved analytical methods have been developed such as polymerase chain reaction techniques[4,5] and immunological assays[6,7] to shorten the total assay time with high specificity. However, these methods have limitations for on-site testing and need expensive reagents. Therefore, there is still a high demand for alternative methods to improve testing efficiency, especially for foodborne pathogens which need rapid detection to ensure food safety and trace foodborne outbreaks, and clinical settings where rapid identification is critical for the diagnosis of the infection source.

Raman spectroscopy is emerging as a promising tool which can be further improved and tailored for the application of analyzing bacteria in a diverse range of fields including food safety, environmental monitoring, and clinical diagnosis.[8–10] It is a powerful analytical technique and has been widely used in the detection of chemical and biological components.[11–13] Raman spectroscopy is a form of vibrational spectroscopy which can be used to build an ultrasensitive platform when coupled with nanomaterials for molecular recognition ability and even single molecule detection.[14] Furthermore, Raman spectroscopy is a non-destructive method so samples will be available for additional investigations. The spectral features that correspond to a wide range of important functional groups can provide essential information about the biochemical constituents of bacterial cells.[15,16] The information can also be used to identify and discriminate bacteria at a species level or strain level. Raman spectroscopy is a powerful technique to detect bacteria in a label-free way and identify the molecular components of bacteria with rapid, sensitive, and non-destructive features.

Bacteria detection is often the initial analysis performed in the areas of food safety, clinical diagnosis, and environmental monitoring. Other bacteria analyses including identification, discrimination, and antibiotic resistance are also necessary. Sample matrices can affect the performance of a method so the preparation of complicated samples is vital for the analysis of microorganisms. New and innovative techniques enable the study of complex samples, such as food matrices and clinical samples. The main emphasis of the review paper is a brief overview of the most recent advancements and the application of Raman spectroscopy for the analysis of bacteria in the past year (2022). The advantages and limitations of these methods and the utilization of Raman spectroscopy for different areas are discussed in the review with the goal to provide an outlook of the most important trends.

2 | RAMAN SPECTROSCOPY FOR THE ANALYSIS OF MICROORGANISMS

Raman spectroscopy utilizes vibrational spectroscopic techniques and measures the inelastic light scattering of the radiation of monochromatic light from a laser source with a vibrating molecule. The interaction of the light with the vibrating molecule yields a spectral shift, also called the "Raman" shift.[17] The basis of Raman spectroscopy is dependent on radiation, or the properties of light scattering, of which there are three types relevant to spectroscopy. The three types of light scattering or photon activity occur simultaneously as shown in Figure 1.

First, there is elastic activity named Rayleigh scattering; most photons are part of this activity, and after interacting with the sample, maintain their initial level of energy.[18] Electrons in the sample molecules are excited, then return to their initial ground state, where there is minimal to no energy lost. This activity occurs more often than the other types of scattering because it is more likely that the electrons will return to the ground state rather than losing or gaining energy. Second, there is inelastic activity named Raman scattering; this phenomenon occurs with roughly every 1 in 10 million photons.[19] This can be further categorized into stokes and anti-stokes scattering, both of which occur as part of Raman scattering, where the photons emitted may lose or gain energy after interacting with the sample in a Raman shift.[18] The Raman shift is a change in wavelength. In stokes scattering, electrons are excited, then move to a higher energy state than the initial ground state, causing photons to lose energy. In anti-stokes scattering, which is more relevant to the analysis, electrons are excited and then move to a lower energy state than the initial ground state, causing photons to gain energy. When considering the interactions between photons and the molecules of the sample, Raman scattering, and stokes scattering in particular, are the relevant types of activity that allow useful data to be obtained from this method.

The inelastic Raman scattering, the Raman shift, and the overall interaction can vary depending on the sample. Nonpolar groups have intense Raman bands. The vibrational patterns given off by the interaction between the laser's photons and the sample's molecular structure are measured by the spectrograph. This is illustrated by a spectral chart of peaks which is unique to the sample. The peak pattern is not only representative of the vibrational patterns of the sample's molecules but also describes the structure of the sample and how it interacts with the photons. It acts as a fingerprint of the molecular structure of the sample.[20,21]

The information from Raman spectra can be used to identify components of the sample, such as singular whole cells, functional groups, or surface proteins. The data is mapped on a chart with the intensity on the Y axis and the Raman shift on the X axis. The peak intensity depends on the unique characteristics of the sample and individual peaks can be separated without overlapping.[22,23] However, there may be interferences that are difficult to differentiate from the target data. The spectrum, whether representing the simple detection of a whole cell or the fingerprint identification of functional group structures and

Mini Review
doi.org/10.1002/ansa.202200066
Chemistry Europe
European Chemical Societies Publishing

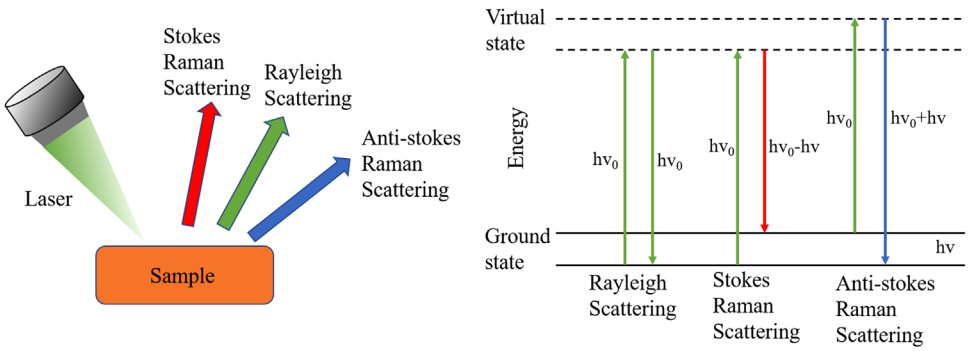

FIGURE 1 Three types of scattering signals that are generated when light interacts with a sample and energy diagram for Rayleigh and Raman scattering processes.

bacterial surface proteins, is used to identify the target analyte present in the sample.

Raman signals are generally weak, but new techniques such as surface-enhanced Raman scattering (SERS) have made signal enhancement possible by using metal colloids or nanostructured metal surfaces. The commonly used SERS substrates are silver or gold nanomaterials.[24] The resulting Raman scattering can be improved by several orders of magnitude by the interaction of small metal particles with the target molecules. Raman spectroscopy for the analysis of bacteria is still facing some limitations regarding sensitivity, accuracy, and interferences from complex samples. Raman spectroscopy can be used to sensitively detect bacteria in samples with low complexity. However, for complex samples like clinical and food samples, the sensitivity needs to be further improved to enable the analysis of low levels of pathogens and infections. High laser power may denature bacteria and change the bacterial structure, potentially affecting the accuracy of the analysis. Therefore, more studies have been focused on the development of novel nanomaterials to improve the sensitivity, more efficient bacteria separation methods to minimize the interference from real samples, and advanced data processing tools to better analyze the raw spectral data. With the use of novel nanomaterials, the Raman intensity of the analysis of biological samples can be increased significantly.[25,26] A Raman spectrometer can also be combined with a microscope. The confocal Raman microscope allows precise measurements and the examination of defined optical sections of two-dimensional (2D) or three-dimensional (3D) mapping of a specific area within a sample.[27,28] Raman spectroscopy can analyze samples in a solid, liquid, or gas state. Biological samples contain different molecules, and a Raman spectrum can offer interpretable and detailed information about all biochemical components in the laser focus of the sample. Raman spectroscopy can be applied for the detection of a single bacterial cell, the monitoring of bacterial cellular response, and the identification of bacterial cellular components.

3 | BACTERIAL DETECTION

Rapid, sensitive, and accurate detection of bacteria continues to be crucial in many areas including food safety, clinical diagnosis, and environmental monitoring. The critical considerations for establishing a detection system include assay time, sensitivity, specificity, cost, size, and sample handling.[29,30] The majority of recent improvements in bacteria detection with Raman spectroscopy are aimed at increasing the sensitivity of the analysis so that the method is capable of detecting bacteria at a low level. It is also desirable and critical to developing an assay which can be used directly on-site. An overview of the recent publications using Raman spectroscopy or SERS for bacteria detection is in Table 1. The table includes substrate, analyte, sample matrix, and limit of detection (LOD).

The SERS technique is often employed to improve sensitivity, in which nanomaterials are used to form an enhanced signal owing to the hot-spot effect of metal particle arrays by surface plasmon resonance. SERS can also improve spectral clarity and reproducibility. Therefore, SERS is more often used in bacterial detection than regular Raman spectroscopy. The two principal strategies for bacterial detection using SERS are label-free and label-based methods. In label-free methods, samples are mixed with the nanoparticles in a suspension or deposited onto the SERS substrate directly, and then the chemical fingerprint spectra of bacterial cells will be acquired.[31,32] An alternative approach is the use of SERS tags to achieve a sensitive detection with the spectra of Raman reporter molecules as label-based methods. The SERS tags are usually composed of nanoparticles modified with capture probes to target bacteria with a Raman reporter molecule.[33–35] This approach has the potential to detect multiple bacteria simultaneously. The examples of these two types of strategies are shown in Figure 2. The recent advances of Raman based bacterial detection are more focused on the development of nanomaterials as novel SERS substrates, the improvement of bacterial separation and concentration methods, the development of portable and simple methods, the combination with other technologies, and the indirect bacterial detection by the analysis of metabolites or nucleic acids.

3.1 | Novel SERS substrates

Various types of novel nanomaterials have been developed as SERS substrates. Noble metal nanoparticles offer a larger hot-spot effect for the detection of various analytes, which enhances the effectiveness

TABLE 1 Recent publications (2022) on bacteria detection using Raman spectroscopy or surface-enhanced Raman scattering (SERS).

Substrate	Analytes	Sample matrix	LOD	Ref.
Au@Ag NPs	*S.* Enteritidis	Botanical drug	52 CFU/ml	31
N/A (Raman spectroscopy)	*E. coli*, *B. cereus*	Bacterial culture	N/A	32
MNPs@Con A/pathogen/TBDP@Raman tags	*E. coli*, *S.* Enteritidis	Milk	101 CFU/ml	33
Au@MNP-WGA tag	*L. monocytogenes*, *C. jejuni*, *S. aureus*	Vegetable juice, fruit juice, and river water	10 CFU/ml	34
ConA-Fe_3O_4@SiO_2 NPs/bacteria/aptamer-modified Au NNPs	*S. aureus*	Serum	11 CFU/ml	35
AuNP/bacteria/Con A+BCNC) conjugates	19 bacterial strains	Bacteria culture	N/A	36
AuNPs	*C. acnes*, *E. coli*, *S. aureus*	Bacteria culture	N/A	37
Au@Ag NPs/slide	*S. aureus*	Milk	6 CFU/ml	38
Au@Ag NPs	*S. aureus*	Milk	0.25 CFU/ml	39
Fe_3O_4@AuNPs@NSP nanosheets	*E. coli*	Buffer	$<10^3$ CFU/ml	40
GO@Au	*S. aureus*, *E.coli* O157:H7, *S.* Typhimurium	Skim milk, carrot juice, grape juice	<10 cell/ml	41
AuNPs	Bacterial molecule (pyocyanin)	Bacteria culture	N/A	42
GO@Au/Ag	*S.* Typhimurium, *E. coli*, *S. aureus*, *L. monocytogenes*	Sputum, urine, whole blood	9 cells/ml	43
Au-NC-NBs	*S. aureus*	Milk, orange juice, human urine, saliva	3 cells/ml	44
AuNPs/bacteria/ConA+BCNC	*E. coli* 8739	Bacterial culture	10^3 CFU/ml	45
Au@Ag NPs	*E. coli*	Bacterial culture	N/A	46
AuNPs/NMPs	*S. aureus*, *E. coli*	Bacterial culture	N/A	47
Fe3O4@Au-Tcp NPs	*S. aureus*	Milk, orange juice	1.09 CFU/ml	48
$Ag_7O_8NO_3$ nanorods	8 bacterial strains, 18 *Salmonella* strains	Bacteria culture	N/A	49
Au@Ag	*E. coli*, *V. anguilarum*, *V. harvevi*, *E. piscicida*, *P. plecoglossicida*	Tap water	10^5 CFU/ml	51
AgNPs@PDMS	*S. aureus*, *S.* Typhimurium, *L. monocytogenes*, *C. difficiles*, *C. perfringens*	Bacterial culture	N/A	52
4-MPBA@AgNPs	*S. paratyphoid*, *B. subtilis*, *E. faecalis*, *S. aureus*	Probiotic beverage, chicken breast	1.35 CFU/ml	53
Au-TPP	*S. aureus*, *S. flexneri*	Pork surface	N/A	50
Au@Ag@SiO_2 nanoparticles	*S. aureus*	Fish, milk	2 CFU/ml	54
Au nanopopcorns	*E. coli*	Mutton	143 CFU/g	55
AuNPs	*E. coli* O157:H7	Lettuce	0.5 CFU/ml	56
AgNPs	*S. aureus*, *E. coli*, *C. indologenes*	Whole blood	3 CFU/ml	57
AuNS@4-MBA@Au@DNA	*S.* Typhimurium	Milk, meat	3-4 CFU/ml	58
Fe3O4@Au NCs	*S. aureus*	Milk	25 CFU/ml	59

of SERS and improves the detection sensitivity and accuracy. The following examples highlight the studies which developed novel SERS substrates and innovative detection platforms based on SERS.

Novel SERS substrates were developed in some studies with a large specific surface area, rich porous structure, or multi- dimensional structure to improve the sensitivity and bacteria capture efficiency, such as nanoparticles,[35–39] nanosheets,[40–42] nanostickers,[43] nanoballoons,[44] and nanopancakes.[45] Li et al. synthesized an all-nanoparticle microcapsule by a layer-by-layer approach as a SERS substrate which enhanced the major bacteria's peak and improved the detection sensitivity.[46] Gold nanoparticles (AuNPs) were adsorbed on the calcium carbonate templates and silver nanoparticles (AgNPs)

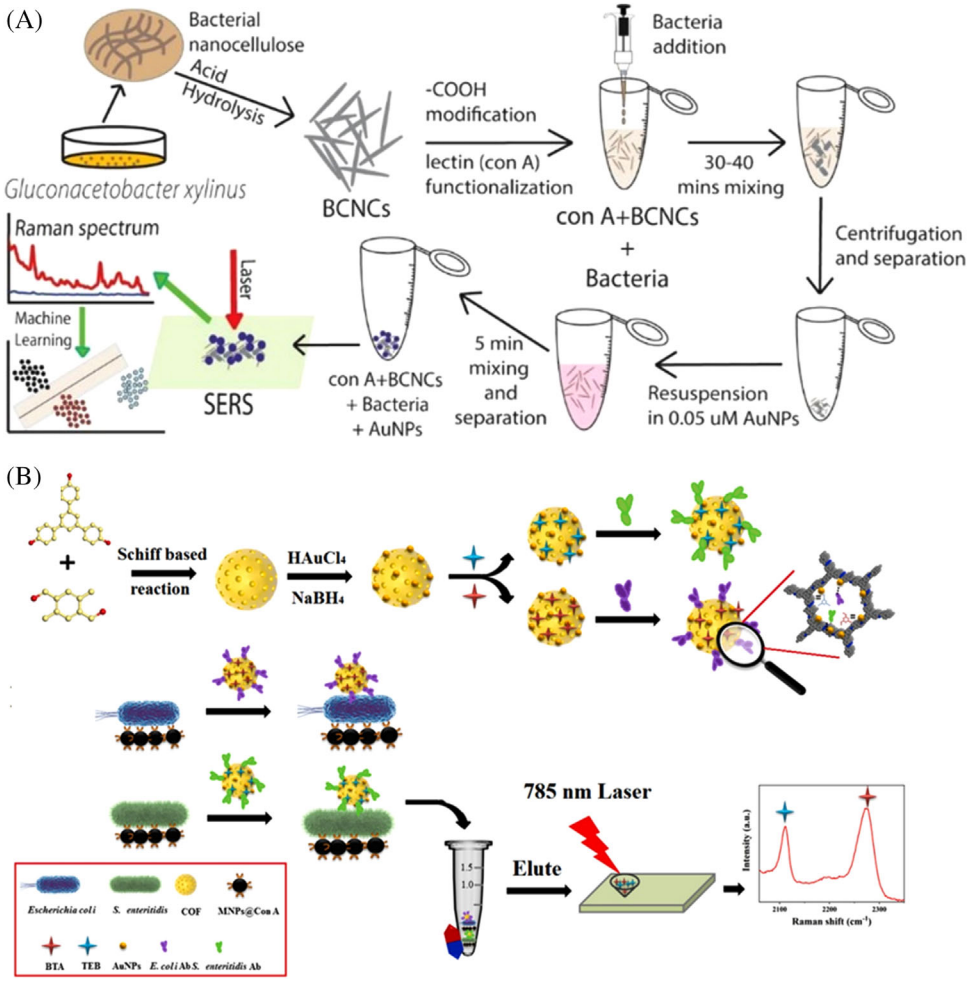

FIGURE 2 The two principal strategies of using surface-enhanced Raman scattering (SERS) for bacteria detection. (A) Label-free method: Samples are mixed with the nanoparticle solution and chemical fingerprint spectra are collected. (B) Label-based method: Magnetic particles are bound with target bacteria for separation, and then SERS tags are added for detection. (Reproduced with permission[33,36]).

were synthesized in situ as layers. The method allowed label-free SERS detection of bacteria and demonstrated 13 additional peaks related to bacteria composition. There are also studies focused on the improvement of the synthesis methods for SERS substrates by using more convenient, easier, and green methods. Chen et al. developed a flexible hybrid substrate by depositing AuNPs on the surface of 2D nanomica platelets (NMPs).[47] The method enables the capture of bacteria of similar polarity due to the concurrently hydrophobic and hydrophilic properties of the nanohybrids. The substrate had high selectivity and enhanced SERS signal intensity. A green synthesis method was developed by Qi et al. for the fabrication of SERS substrates by reduction of natural cationic polysaccharide chitosan to compose AuNPs.[48] It is an eco-friendly, cheap, and simple method for the synthesis of sensitive SERS substrates. Chen et al. fabricated nanoporous silver nanorods with easily controlled length and thickness through a simple chemical reduction.[49] The substrate was used for the bacteria detection of 26 strains. The nanorods had a simple fabrication process and high performance as a promising SERS substrate for sensitive bacteria detection.

3.2 | Bacteria separation and concentration

Sample preparation is an important step for the bacterial analysis in order to obtain an accurate and sensitive result. The real sample matrices that most research works on for bacterial analysis with Raman spectroscopy are liquid samples as shown in Table 1. That is mainly due to the relatively easier sample preparation for liquid samples. While for the analysis of the whole solid samples, the samples need to be mixed with the diluent buffer and homogenized with a stomacher. Then the liquefied sample will be used for the analysis of bacteria.[31] For the surface of the solid samples, a tape or swab can be used to collect the sample and to be directly analyzed by Raman spectroscopy.[50] After the homogenization of the samples, an efficient and specific separation of bacteria from complex samples can still be challenging. The food particles and other components in the sample matrices might interfere with the reaction and affect the final analysis results. Therefore, an effective method to separate bacteria from complex samples is important to enhance the accuracy of the

detection. The bacteria separation is often combined with bacteria concentration, thus the concentration of target bacteria will be increased and the sensitivity of methods will also be improved. Various studies and different strategies have been developed to address these issues.

The immunomagnetic separation and filtration methods attract more attention and new technologies have been incorporated into these methods with novel nanomaterials and bio-recognition elements. Magnetic beads are useful tools for specific and fast separation and concentration of analytes from complex samples by applying a magnet and are ideal for on-site detection. The use of nanomaterials is often combined with magnetic separation for the isolation of targeted bacteria. Magnetic iron-oxide (Fe_3O_4) nanoparticles are often combined with noble metal nanoparticles such as AuNPs[40] and Au@Ag core-shell bimetallic nanoparticles[51] to enable in situ bacteria separation and the SERS hotspot effect. The magnetic SERS substrate was applied for the detection of *Escherichia coli* (*E. coli*) in real aqueous samples and the results demonstrated the sensitivity was increased by approximately two times after magnetic separation.[40] Filtration is an alternative for bacteria separation and concentration. Zhu et al. reported a study that developed a multi-functional AgNPs@PDMS (polydimethylsiloxane) multi-hole filter membrane chip, allowing the concentration of bacteria on the membrane for SERS detection.[52] The SERS signal was greatly enhanced. The method offered a simple and efficient sample preparation for microbiological sampling onto SERS substrates and was applied for the detection of several different foodborne pathogens. However, the binding between nanoparticles and bacteria was not specific in these studies. Biorecognition elements such as antibodies and aptamers are often used to specifically capture target bacteria when developing SERS based bacteria detection. Jin et al. developed an aptamer-based SERS method for the rapid detection of *Salmonella* Enteritidis (*S.* Enteritidis) by using a portable Raman instrument.[31] 4-mercaptobenzoic acid (4-MBA) served as a Raman reporter molecule and was self-assembled on the surface of Au@Ag NPs. The free aptamers in the solution changed the SERS signal and were correlated to the bacteria concentration. The approach showed high specificity to *S.* Enteritidis with an LOD of 53 CFU/ml in a drug sample. Cell imprinting mapping technique is a novel approach to specifically recognize and capture bacteria. It has been successfully incorporated for the specific detection of different bacteria using SERS in both liquid and solid food samples by Yang et al.[53] Molecularly imprinted polymers were used in a study to specifically detect *E. coli* and *Bacillus cereus* (*B. cereus*) by SERS.[32] Magnetic separation can be combined with biorecognition elements to enhance the sensitivity and specificity of detection methods. A study synthesized an interference-free Raman tag based on covalent organic frameworks which was modified with antibodies.[33] The target bacteria were first captured by lectin functionalized magnetic nanoparticles for magnetic separation and then formed a sandwich-like composite with the antibody modified Raman tags. With the use of two specific antibodies, the method was applied for the multiplex detection of *E. coli* and *S. Enteritidis* in milk samples and achieved an LOD of 101 CFU/ml.

3.3 | Method simplicity and portability

In addition to sensitivity and specificity, the ease of use and simplicity of a detection method is also critical. The development of portable methods for on-site use is beneficial for clinical and food safety testing of real samples. Additionally, commercially available portable Raman spectroscopy can be easily used for in-field testing. Zhao et al. developed a hands-on detection technique by synthesizing a flexible SERS substrate through the precipitation of AuNPs on an inexpensive tobacco packaging paper (TPP).[50] The paper based enhanced substrate allowed a flexible SERS platform with in situ sample collection by directly swabbing onto the sample. However, the method is limited to the detection of surface bacteria of a sample. Even though the method was applied for the analysis of two different species, the mixture of bacteria species still needs to be tested to determine the results accuracy. Overall, the developed approach is a promising strategy for the on-site screening of microbial contamination of sample surfaces when combined with portable Raman spectroscopy. A similar study fabricated a film for bacteria detection but in aqueous samples.[54] A vancomycin grafted PDMS film was created for capturing bacteria in the solution. SiO_2-coated Au@Ag nanoparticles modified with an aptamer were used for SERS signals. An LOD of 2 CFU/ml was achieved for *Staphylococcus aureus (S. aureus)* in food samples. The approach has considerable ease of use and can be applied in more food matrices. Gold nanomaterials modified tape and array were developed to easily collect solid surface samples for bacteria detection. Another interesting SERS based method that can be used to easily capture bacteria was designed by Wang et al.[55] The study developed a plasmonic hydrogel microneedle array patch coated with gold nanopores that were modified with specific aptamers for *E. coli*. The developed substrate enabled both the extraction of bacteria from solid samples and SERS detection. The method was applied to the analysis of *E. coli* on mutton samples. The approach showed a high specificity and an LOD of 143 CFU/g. However, liquid samples should be tested for future studies. The current design of the method is limited to the sample area that the substrate attached to, while it is not available for large samples or the internal part of the samples. Overall, the strategy still provided an innovative way to simplify the sample preparation requirements and has high potential for in-field microbial analysis.

3.4 | Combination with other technologies

Raman spectroscopy can be used with other technologies to improve the performance of the assay, such as lateral flow assays (LFAs) and microfluidics. Several studies combined SERS with lateral flow immunoassay for the detection of different bacteria.[34,41,43] Lateral flow assays offer the advantages of low costs, real-time analysis, and portability. When combined with SERS, the sample preparation step can be simplified, and the sensitivity can be improved. Multi-dimensional membrane-like SERS tags were developed to act as the SERS label with a larger surface area for quickly capturing targeted

Mini Review
doi.org/10.1002/ansa.202200066
Chemistry Europe
European Chemical Societies Publishing

bacteria and for improving the SERS hotspot effect. For the study of Wang et al,[43] GO@Au/Ag nanostickers were labeled with two Raman reporter molecules for the simultaneous analysis of four bacteria in two test lines with an LOD of 9 CFU/ml within 20 min. The method was applied in real clinical samples (human urine and blood). The strategy significantly reduced total assay time for SERS detection and increased the detection throughputs of LFA methods. Integration of SERS tags into a microfluidic dielectrophoretic sensor led to a very low detection limit for complex samples.[56] Chen and coworkers combined SERS detection with microfluidics for a system with the capability of bacteria pre-concentration, bacteria identification, and antibiotic susceptibility study.[57] A hybrid electrokinetic mechanism allowed for the thousand-fold concentration of bacteria. The SERS detection was conducted on a microchip for whole blood samples with an LOD of 3 CFU/ml.

3.5 | Indirect bacteria detection

Several studies measured the signaling molecules (metabolites) or the nucleic acids of bacteria with SERS to determine the bacteria level rather than detecting the target bacteria whole cells directly. Kim and coworkers designed a paper-based 3D SERS substrate, enabling the Au electrodeposition and the bacteria metabolites detection simultaneously without pretreatment steps.[42] The hydrogel skin of the substrate excluded the attachment of macromolecules from the culture which avoided the interference. The strategy resulted in high amplification of the Raman signal intensity. A study developed a SERS-based CRISPR/Cas assay on microfluidic paper.[58] Single-stranded DNAs of the target bacteria pulled down the SERS nanoprobes. The degree of aggregation of SERS nanoprobes was correlated to the concentration of the bacteria. The approach was able to detect *Salmonella* Typhimurium (*S.* Typhimurium) with an LOD of 3–4 CFU/ml in milk and meat samples.

Bacterial detection can also be achieved simultaneously with bacterial inactivation by the development of a novel SERS substrate. A research group developed a SERS substrate of triple-functional Au–Ag-stuffed nanopancakes (AAS-NPs) for bacteria capturing, detection, and inactivation.[45] 4-mercaptophenylboronic acid served as a SERS tag and Raman reporter molecule. A sandwich structure of bacteria/SERS tags/AAS-NPs was fabricated for sensitive SERS sensing. Multiple bacteria including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (*P. aeruginosa*) were captured and analyzed in human blood samples with an LOD of 7 CFU/ml. The expressive Ag+-accelerated releasing capability allowed for the antibacterial effects. Another study developed a strategy using Concanavalin A conjugated Fe_3O_4@SiO_2 nanoparticles for the magnetic separation of bacteria, then formed a sandwich composite with aptamer modified Au nano-bridged nanoparticles. The magnetic nanoparticles also had a photothermal elimination effect on bacteria.[35,59]

The sensitivity of bacterial detection methods is always important. The development of novel nanomaterials for SERS substrate and bacterial separation and concentration to achieve a lower LOD will still be a trend. Further research will continue focusing on the nanomaterials with large surface areas, multi-dimensions, and simple and green synthesis methods. The combination with other technologies to improve the sensitivity and simplify the detection steps have attracted more attention for in-field applications.

4 | BACTERIA IDENTIFICATION AND DISCRIMINATION

While a rapid and accurate detection of the bacteria is critical, the identification and discrimination of bacteria present in the sample is also important information. It is essential to rapidly determine the species or strain of bacteria for a disease in clinical diagnoses and for foodborne outbreak tracking. The variation in the biochemical composition of bacterial cell membranes allows for the identification of bacteria species with Raman spectroscopy due to the different vibrational modes of biomolecules. The main advantage of Raman spectroscopy is its capability for providing specific fingerprinting spectra, enabling the selective discrimination between different kinds of bacteria genera, species, and even strains. However, the differences among varied bacteria can be very minor or subtle. It is still challenging to achieve strain level differentiation due to the similar Raman profiles. Thus, it is essential to develop novel techniques to enhance the Raman signal, to amplify the differences among bacterial strains, and to better interpret Raman spectra. Different strategies have been investigated for better identification and discrimination of varying species. Detailed information about the bacteria identification and discrimination by Raman spectroscopy is summarized in Table 2 with representative publications cited in the table.

4.1 | Advanced data processing methods

Advanced statistical analysis and data processing techniques are critical for studies targeted at the differentiation and identification of bacterial strains to interpret the raw Raman spectral data. A variety of methods have been developed to analyze high-dimensional data, such as chemometrics tools and machine learning algorithms.[60] Chemometrics, a multivariate statistical analysis, can reduce multidimensional information to independent variables. The analysis can be unsupervised or supervised models. Traditional linear classification models are challenging when the Raman spectra of bacteria are highly similar. Different multivariate data analysis methods have been developed for the identification and discrimination of bacteria with Raman spectroscopy, such as principal component analysis (PCA) in Figure 3A,[61–64] partial least square discriminant analysis (PLS-DA),[63,65,66] and discriminant function analysis (DFA).[64] These methods have been applied to discriminate waterborne pathogen species in drinking water,[62] to discriminate bacterial strains with different biofilm forming abilities,[63] to identify bacteria and yeast species in blueberries (Figure 3B),[64] and to differentiate common microbes in urine.[67] PCA is a multivariate analysis and the most commonly used method to analyze Raman spectra.

Mini Review
doi.org/10.1002/ansa.202200066

TABLE 2 Recent publications (2022) on bacteria identification and discrimination using Raman spectroscopy or surface-enhanced Raman scattering (SERS).

Substrate	Analytes	Sample matrix	Data processing	Ref.
AgNPs	*E. faecalis*, 3 types of *E. coli*	Bacterial culture	PCA	61
AgNPs	*C. parvum*, *E. coli*, *S. aureus*	Bacterial culture	PCA, HCA	62
AgNPs	15 bacterial strains	Bacterial culture	PCA, PLS-DA	63
AgNPs	*S. hominis*, *S. cohnii*, *S. enterica*, *L. mesenteroides*, *C. santamariae*,	Blueberry	PCA	64
N/A (Raman spectroscopy)	*B. subtilis*, *Salmonella enterica*	Drug sample	PLS-DA	65
Au@Ag NPs	*S. aureus*, *E. coli*, *L. monocytogenes*	Bacterial culture	PLS-DA	66
N/A (Raman spectroscopy)	20 bacteria species	Urine	PCA, SVM	67
AgNPs	Multiple bacterial strains	Bacterial culture	K-means clustering, agglomerative nesting (AGNES), CNN	68
AuNPs	*S.* Typhimurium, *S.* Enteritidis, *S. paratyphoid*	Bacterial culture	CNN	69
N/A (Raman spectroscopy)	*S.* Enteritidis, *S.* Typhimurium, *S. derby*	Bacterial culture	CNN	70
N/A (Raman spectroscopy)	*Porphyromonas gingivalis (Pg)*, *Fusobacterium nucleatum (Fn)*, *Aggregatibacter actinomycetemcomitans (Aa)*	Bacterial culture	Extra trees, AdaBoost, gradient boosting, LDA, SVM, multi-layer perceptron, passive-aggressive classifier, quadratic discriminant analysis	71
N/A (Raman spectroscopy)	21 species of microorganisms	Bacterial culture	PCA, SVM	72
N/A (Raman spectroscopy)	11 bacterial mutants of E. coli MDS42	Bacterial culture	Artificial neural network, SVM	73
AgNPs	*C. perfringens*, *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *P. pabuli*	Garlic powder	k-nearest neighbour (KNN), least square LS-SVM	74
N/A (Raman spectroscopy)	*E. coli* O157:H7, *V.* parahemolyticus, *S.* Typhimurium	Bacterial culture	GAN-SVM	75
Ag-based	*E. coli*, *S. epidermidis*	Milk	Dual-branch wide-kernel network (DualWKNet)	76
AgNPs	*S. aureus*, *B. cereus*	Bacterial culture	PCA	77
Au@Ag NPs	8 bacterial species	Bacterial culture	Ward's HCA	78
AgNPs-SASP	*C. perfringens*, *B. subtilis*, *B cereus*	Bacterial culture	HCA, LDA	79

It can be combined with hierarchical cluster analysis to further discriminate bacteria species.

Machine learning algorithms were combined with Raman spectroscopy for speeding up the identification and obtaining a high differentiation accuracy. The principle of bacteria identification and discrimination by Raman spectroscopy with machine learning for data processing is shown in Figure 3C. In particular, convolutional neural networks (CNN)[68–70] and support vector machine (SVM)[67,71,72] have received widespread attention in Raman spectra analysis. SVM is an optimization-based machine learning technique which increases the data dimensionality to convert a nonlinear classification into a linear one. CNN is suitable for multi-classification analysis for in-depth mining and interpretation of Raman spectra. A study compared two machine learning models (an artificial neural network and a SVM) for the discrimination between 11 bacterial mutants of *E. coli* with Raman spectroscopy.[73] Both models were found to have similarly high sensitivity, specificity, and accuracy. Another study compared multiple machine learning algorithms to discriminate clinically important pathogens, and CNN achieved the highest prediction accuracy.[68] There are also other models frequently used in the Raman spectra analysis such as least squares SVM (LS-SVM)[74] and K-means clustering.[68] LS-SVM is an extension of standard SVM and uses a least square linear system as the loss function.

Machine learning algorithms normally rely on a large amount of spectral data to train the model to improve the differentiation efficiency. However, large volume data acquisition for bacteria identification not only involves massive work but is also technically difficult in many cases. In order to overcome the cumbersome operation, a study utilized generative adversarial network and multiclass SVM (GAN-SVM) to classify three pathogenic bacteria.[75] The GAN model was able

Mini Review
doi.org/10.1002/ansa.202200066
Chemistry Europe
European Chemical Societies Publishing

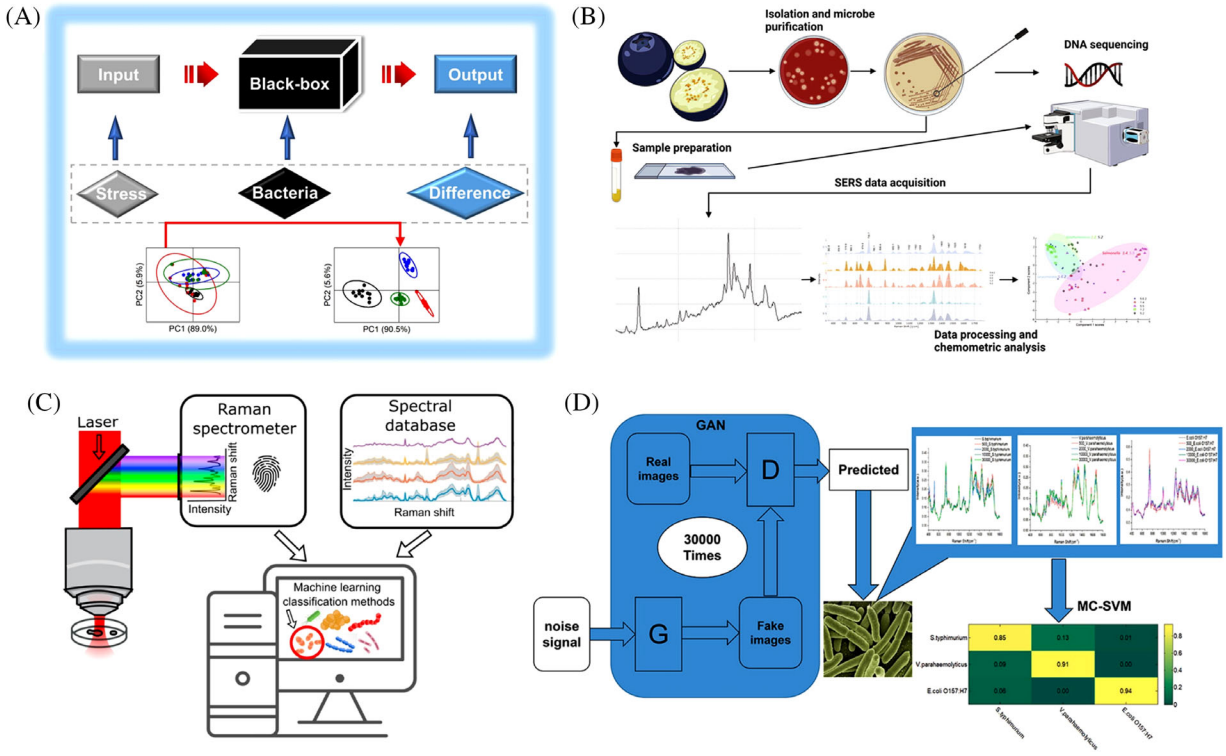

FIGURE 3 Different data processing techniques cited within this review. (A) Principal component analysis (PCA) for bacteria differentiation with signal enhancement by environmental stress. (B) PCA and discriminant function analysis (DFA) for the characterization of bacteria and yeasts. (C) Principle of microbial identification by Raman spectroscopy with machine learning for data processing. (D) Generative adversarial network and multiclass support vector machine (GAN-SVM) model for bacteria discrimination with a small amount of data samples. (Reproduced with permission[58,61,67,75]).

to integrate all the data and achieve powerful embedding from the neighboring nodes. It can achieve good results with a small number of data samples. SVM enabled better recognition and classification capabilities than traditional methods, especially when dealing with large amounts of similar spectral information. The specific spectral peaks obtained from the classification results allowed the identification of multiple bacterial species. The GAN-SVM model shows better resource efficiency and classification results as a promising tool for Raman spectroscopy for bacteria identification. Another novel deep learning model, dual-branch wide-kernel network (DualWKNet) offered a simpler, faster, and effective classification of two common bacteria without any separation procedures with accuracy up to 98%.[76] These developed methods require a short data acquisition time and small amounts of training data.

4.2 | SERS substrate modification

In addition to the advanced data processing tools, other strategies have been developed to improve the discrimination ability for different species. Alternative strategies were on the modification of SERS substrates. The surface modification of SERS nanomaterials resulted in the additional difference of the SERS fingerprints to improve the differentiation efficiency. One study modified AgNPs with multiple chemicals to provide additional dimensionality of the substrate.[77] The modified SERS substrate was able to distinguish different strains of *B. cereus* simply by PCA, while the bare AgNPs only separated *S. aureus* and *B. cereus*. Tian et al. developed a biofunctional nanoparticle array through self-assembly of noble metal nanoparticles into dense 2D thin arrays at liquid-liquid interfaces.[78] The nanoarrays exhibited high sensitivity and reproducibility and were able to differentiate 8 different bacteria species with a high signal-to-noise ratio. Another similar study developed a sensitive 2D AgNPs self-assembled solid-phase (AgNPs-SASP) for the SERS substrate.[79] The substrate enhanced the effect of the SERS signal and three bacterial spores were identified.

4.3 | Environmental stress for signal amplification

External environmental stressors can also be used as the input to treat bacteria to amplify the differences among varied species and diversify the spectral differences. Bacteria have multiple complex regulatory networks to counteract stress and repair damage, resulting in distinct responses for different types of bacteria even with the same environmental stress. In one study conducted by Liu and coworkers, the SERS spectra were shown to be amplified by environmental stress (ultraviolet light, ethanol, and ultrasound) to achieve bacteria discrimination.[61] The SERS profiles also provided molecular difference

information among various bacteria under the external stimulus. After the stress treatment, strain levels were discriminated with PCA. By placing bacteria under different environmental stress, the bacteria can be more easily identified and classified with greater reliability. Raman spectroscopy enables the rapid identification and discrimination of bacteria. With advanced analysis tools, even closely related bacterial species can be distinguished. This is highly important for the prevention of foodborne outbreaks and the spread of pathogens.

The research on bacterial differentiation and classification is mainly focused on two areas, the capability to determine the minor differences among samples, and the strategy to amplify the difference. Therefore, the advanced data processing tools attract more and more interest in order to differentiate strains with very minor differences. Environmental stress will be useful to amplify the signal to achieve a more accurate bacteria classification.

5 | ANALYSIS OF BACTERIA ANTIBIOTIC RESISTANCE AND SUSCEPTIBILITY

The antibiotic resistance of microorganisms is increasing due to the over-use of antibiotics and has become a significant issue for disease control and human health. Antimicrobial susceptibility testing is often performed to quantitatively determine the antimicrobial effects of a drug on pathogens. Current methods either require long cultivation periods or expensive reagents. A rapid and sensitive method to test the antibiotic sensitivity of a pathogen is essential for the prevention of the spread of antibiotic resistance, the management of antimicrobial drugs, and understanding mechanisms of the antibiotic susceptibility of bacteria. Additionally, the identification of the antibiotic resistant bacteria is highly important for efficient treatment decisions and epidemiological studies in clinical settings. Thus, there is a high demand to develop rapid and reliable methods for antimicrobial susceptibility testing and the identification of antibiotic resistant bacteria to facilitate effective and prompt disease treatment. Raman spectroscopy is a novel technique with high potential for clinical applications. It provides the chemical fingerprint of bacterial cells with minimal sample preparation. Different bacteria vary in their chemical composition and macromolecular constituents on cell membranes. When the cells are exposed to antibiotics, antibiotic resistant and sensitive bacteria will undergo different metabolic pathways and change cellular chemical composition. Raman spectroscopy offers a rapid and quantitative analysis of bacterial antibiotic resistance which is highly important for the prevention and treatment of diseases and understanding antibiotic resistance mechanisms.

5.1 | Different bacterial responses during antibiotics exposure

SERS has been successfully applied to analyze the different responses of antibiotic resistant and sensitive bacteria during exposure to antibiotics to assess the antibiotic susceptibility of bacteria.[80] It has been applied to differentiate the antibiotic-resistant and susceptible *E. coli* strains,[81–83] *S.* Typhimurium,[84] *B. cereus*,[82,85] *S. aureus*,[86] and carbapenem sensitive and resistant *Klebsiella* strains from clinical samples.[87] In addition to the instant one-time treatment of antibiotics, the antibiotic susceptibility testing was conducted over a long period such as three months, or 21 days in some studies, to investigate the dynamic molecular changes in the drug-resistant strains.[84,88] The bacteria responded to antibiotic stimulation rapidly and developed different degrees of drug resistance gradually.[88] SERS was used to record the molecular changes that accumulated in the antibiotic-resistant bacterial strains. Additionally, SERS has been used for the antimicrobial susceptibility testing of a drug which is a key measure to enable appropriate antimicrobial treatment in clinical labs. The minimum inhibitory concentration (MIC) often needs to be determined during the test in which bacteria are exposed to an antibiotic with a series of concentrations and the responses are measured to determine the MIC. Saleem and coworkers characterized the antimicrobial properties of two drugs against *Bacillus subtilis* (*B. subtilis*).[89] Significant changes of the signal intensity were detected by SERS following the application of antibiotics. The SERS spectra features changed consistently with the increased antibiotic concentration.

The identification of antibiotic resistant and sensitive bacteria can be achieved with or without exposure to antibiotics. It was found that the antibiotic resistant clinical *E. coli* strains had a higher nucleic acid/protein ratio than the antibiotic sensitive ones.[90] Without exposure to antibiotics, the analysis methods for Raman spectra are more important due to the very minor differences between bacteria. The treatment of antibiotics can amplify their differences because of their varied responses to antibiotics. The methods used to analyze the Raman spectra for antibiotic resistance studies include chemometrics methods such as PCA and PLS-DA,[81–84,89,90] and machine learning algorithms such as CNN and SVM.[87,90,91] SERS or Raman spectroscopy for the analysis of bacteria antibiotic resistance and susceptibility with different data processing methods was shown in Figure 4. Machine learning methods have been used to successfully predict the resistance and sensitivity of *Klebsiella pneumonia* to carbapenem with a CNN algorithm.[87,91] The study conducted by Lu et al.[91] determined that the CNN model simplified the classification system for Raman spectroscopy and provided higher accuracy for the identification of antibiotic resistant strains when compared with the SVM and logistic regression models.

5.2 | Combination with other technologies

SERS can be combined with other techniques to simplify the assay procedure or improve the accuracy. Gukowsky and coworkers compared three different sample preparation methods and found that in situ mapping of bacterial cells on a filter membrane provided the highest signal intensity and most accurate results regarding the differentiation between antibiotic resistant and sensitive *E. coli*.[83] Lin et al. combined SERS with microfluidics that generated a concentration gradient for antibiotics along lateral microwells so the bacteria could be

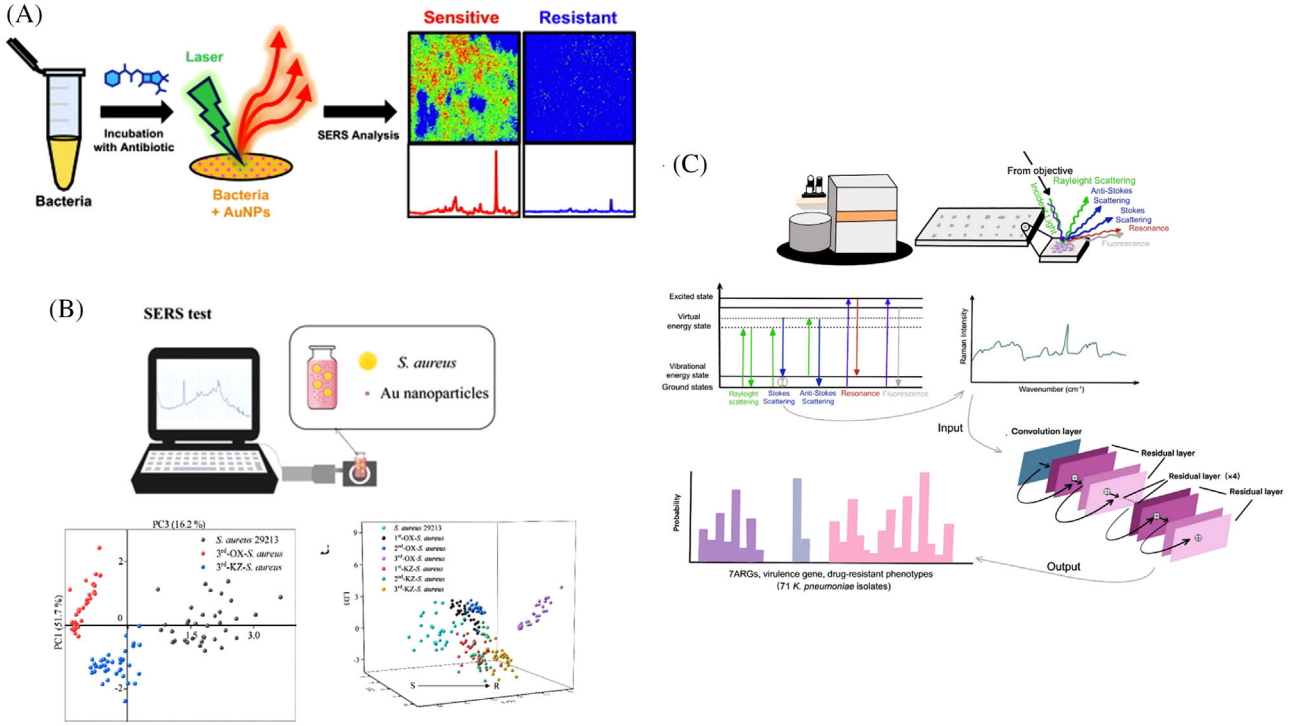

FIGURE 4 Surface-enhanced Raman scattering (SERS) or Raman spectroscopy for the analysis of bacterial antibiotic resistance and susceptibility with different data processing methods. (A) SERS-based filter mapping for testing bacteria antibiotic sensitivity, (B) SERS for dynamic monitoring of bacteria antibiotic resistance by principal components-linear discriminant analysis (PC-LDA), and (C) Raman spectroscopy for antibiotic resistance analysis with the construction of ResNet taxonomic model. (Reproduced with permission[83,88,91]).

encapsulated for antibiotic treatment and subsequent SERS analysis.[92] The method greatly simplified the sample preparation procedure and shortened the assay time. The whole analysis was performed on a chip with only 20 μL of bacteria solution within 5 h. In addition, portable Raman spectroscopy can also be used for the analysis of bacterial antibiotic susceptibility, which makes it possible for future use in low-resource settings.[84,85,88]

Stable isotope labelling is often used in antibiotic susceptibility analysis because the metabolic responses of antibiotic-resistant and susceptible bacteria are varied. Labelling with heavy water is usually performed to indicate the general metabolic activity of active cells. The response of metabolically active bacteria to antibiotics can be differentiated from those of inactive cells. With stable isotope labelling, Raman spectroscopy can further demonstrate the responses of metabolically active cells to antibiotics regardless of the cultivation ability of the cells. A study identified multiple antibiotic-resistant bacteria in chicken faeces and maggots using Raman spectroscopy.[93] Samples were incubated in D_2O-containing antibiotics and then C-D Raman bands were measured to distinguish bacterial strains. Similar studies were conducted for blood cultures[94] and clinical strains.[95,96]

Similar to antibiotics, there are studies that investigated bacterial responses to environmental stressors,[97,98] heavy metals, and herbicides.[99] These studies are also helpful for the understanding of bacterial inactivation mechanisms and the effect of different stressors or chemicals on bacterial cells. SERS has shown to be a promising method to determine the stress response of bacteria at the molecular level. Raman spectroscopy was proved to have the potential to provide accurate and fast identification of antibiotic-resistant bacteria and analyze the responses to antibiotics based on the studies above. It offers a rapid alternative to current antibiotic susceptibility testing methods.

6 | SUMMARY AND OUTLOOK

Raman spectroscopy is a promising technique that can be applied for bacteria detection, bacteria identification and discrimination, and bacteria antibiotic susceptibility testing. Raman spectra provide complementary information about the biochemical properties of bacterial cells. This review highlighted the recent application of Raman spectroscopy for bacteria analysis in a wide range of areas including food safety, clinical testing, and environmental monitoring. The improvements and development of Raman spectroscopy-based methods have been focused on the fabrication of novel SERS substrates, the development of advanced spectra interpretation tools, the study of more efficient sample preparation methods, and the involvement of other techniques for better assay performance.

Utilizing advanced data processing methods will make it possible to better interpret Raman spectra and identify minor differences in a biological sample. It could be a problem to compare the results generated by different data processing tools. Future works should address this issue by developing a universal tool or database for more reliable results. The application of Raman spectroscopy in practical settings is

also one of the future trends. Currently, it is still challenging to analyze the bacteria in complicated environmental, clinical, or food samples and large volume samples due to the interference from the sample matrices. The development of sample preparation techniques with a simple procedure and short time that are compatible with Raman spectroscopy needs further investigation. The combination of other novel techniques with Raman spectroscopy to study microorganisms is still developing and should provide new advances for a portable system for in-field testing. All of these are important considerations for improving Raman spectroscopy for the analysis of microorganisms.

Overall, the studies presented in this review demonstrate that the Raman spectroscopy technique is capable of rapidly detecting, identifying, and discriminating bacteria, especially combined with effective bacterial separation and concentration strategies. Raman spectroscopy has great potential in the field of bacteria analysis. The application of Raman spectroscopy will continue to grow in a broad range of areas with the development and improvement of innovative technologies.

AUTHOR CONTRIBUTIONS

Linsey Rodriguez: lead writing and editing. Zhiyun Zhang: writing and editing. Danhui Wang: conceptualization, guidance, writing and editing

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Research Enhancement Program (REP) at Texas Woman's University.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created.

REFERENCES

1. Váradi L, Luo JL, Hibbs DE, et al. Methods for the detection and identification of pathogenic bacteria: Past, present, and future. *Chem Soc Rev*. 2017;46(16):4818-4832.
2. Law JW, Ab Mutalib N, Chan K, Lee L. Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Front Microbiol*. 2015;5:770.
3. Gracias KS, McKillip JL. A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Can J Microbiol*. 2004;50(11):883-890.
4. Vinayaka AC, Ngo TA, Kant K, et al. Rapid detection of salmonella enterica in food samples by a novel approach with combination of sample concentration and direct PCR. *Biosens Bioelectron*. 2019;129:224-230.
5. Jian C, Luukkonen P, Yki-Järvinen H, Salonen A, Korpela K. Quantitative PCR provides a simple and accessible method for quantitative microbiota profiling. *PLoS One*. 2020;15(1):e0227285.
6. Seo S, Lee Y, Jeon JH, et al. Highly sensitive detection of a biothreat pathogen by gold nanoparticle-based oligonucleotide-linked immunosorbent assay. *Biosens Bioelectron*. 2015;64:69-73.
7. Wu W, Li J, Pan D, et al. Gold nanoparticle-based enzyme-linked antibody-aptamer sandwich assay for detection of salmonella typhimurium. *ACS Appl Mater Interfaces*. 2014;6(19):16974-16981.
8. Sun Y, Tang H, Zou X, Meng G, Wu N. Raman spectroscopy for food quality assurance and safety monitoring: A review. *Curr Opin Food Sci*. 2022;47:100910. https://www.sciencedirect.com/science/article/pii/S2214799322001126. https://doi.org/10.1016/j.cofs.2022.100910
9. Cui D, Kong L, Wang Y, Zhu Y, Zhang C. In situ identification of environmental microorganisms with Raman spectroscopy. *Environ Sci Ecotechnol*. 2022:100187.
10. Terry LR, Sanders S, Potoff RH, Kruel JW, Jain M, Guo H. Applications of surface-enhanced Raman spectroscopy in environmental detection. *Anal Sci Adv*. 2022;3(3-4):113-145. https://doi.org/10.1002/ansa.202200003
11. Zhang Z, Xia M, Ma C, et al. Rapid organic solvent extraction coupled with surface enhanced Raman spectroscopic mapping for ultrasensitive quantification of foliarly applied silver nanoparticles in plant leaves. *Environ Sci Nano*. 2020;7(4):1061-1067.
12. Wang D, Yang J, Pandya J, et al. Quantitative age grading of mosquitoes using surface-enhanced Raman spectroscopy. *Anal Sci Adv*. 2022;3(1-2):47-53.
13. Wang D, Du X, He L. Investigation of the impact of a pesticide adjuvant on dimethoate persistence, penetration, and stability on apples using surface-enhanced Raman spectroscopy. *ACS Agric Sci Technol*. 2022;2(4):788-795.
14. Yılmaz D, Günaydın BN, Yüce M. Nanotechnology in food and water security: On-site detection of agricultural pollutants through surface-enhanced Raman spectroscopy. *Emerg Mater*. 2022:1-28.
15. Allakhverdiev ES, Khabatova VV, Kossalbayev BD, et al. Raman spectroscopy and its modifications applied to biological and medical research. *Cells*. 2022;11(3):386.
16. Pahlow S, Meisel S, Cialla-May D, Weber K, Rösch P, Popp J. Isolation and identification of bacteria by means of Raman spectroscopy. *Adv Drug Deliv Rev*. 2015;89:105-120.
17. Kudelski A. Analytical applications of Raman spectroscopy. *Talanta*. 2008;76(1):1-8.
18. Larkin P. *Infrared and Raman spectroscopy: Principles and spectral interpretation*. Elsevier; 2017.
19. Nivaz SR, Geethalakshmi R, Lekshmi GS, Surendhiran D, Hussain CM, Sirajunnisa AR. Utilization of Raman spectroscopy in nanomaterial/bionanomaterial detection. In: *Handbook of* Microbial Nanotechnology. Elsevier; 2022:145-156.
20. Das RS, Agrawal YK. Raman spectroscopy: Recent advancements, techniques and applications. *Vib Spectrosc*. 2011;57(2):163-176.
21. Petry R, Schmitt M, Popp J. Raman spectroscopy—a prospective tool in the life sciences. *ChemPhysChem*. 2003;4(1):14-30.
22. Kuhar N, Sil S, Verma T, Umapathy S. Challenges in application of Raman spectroscopy to biology and materials. *RSC Adv*. 2018;8(46):25888-25908.
23. Pence I, Mahadevan-Jansen A. Clinical instrumentation and applications of Raman spectroscopy. *Chem Soc Rev*. 2016;45(7):1958-1979.
24. Schlücker S. Surface-Enhanced Raman spectroscopy: Concepts and chemical applications. *Angew Chem Int Ed*. 2014;53(19):4756-4795.
25. Langer J, Jimenez de Aberasturi D, Aizpurua J, et al. Present and future of surface-enhanced Raman scattering. *ACS nano*. 2019;14(1):28-117.
26. Lin X, Cui Y, Xu Y, Ren B, Tian Z. Surface-enhanced Raman spectroscopy: Substrate-related issues. *Anal Bioanal Chem*. 2009;394(7):1729-1745.
27. da Costa SG, Richter A, Schmidt U, Breuninger S, Hollricher O. Confocal Raman microscopy in life sciences. *Morphologie*. 2019;103(341):11-16.
28. Rzhevskii A. The recent advances in Raman microscopy and imaging techniques for biosensors. *Biosensors*. 2019;9(1):25.
29. Hameed S, Xie L, Ying Y. Conventional and emerging detection techniques for pathogenic bacteria in food science: A review. *Trends Food Sci Technol*. 2018;81:61-73.

30. Nnachi RC, Sui N, Ke B, et al. Recent progress on biosensors for rapid detection of bacterial pathogens in water, food and environment. *Environ Int*. 2022:107357.
31. Jin L, Wang S, Shao Q, Cheng Y. A rapid and facile analytical approach to detecting salmonella enteritidis with aptamer-based surface-enhanced Raman spectroscopy. *Spectrochim Acta Part A*. 2022;267:120625. https://www.sciencedirect.com/science/article/pii/S1386142521012026
32. Bräuer B, Werner M, Baurecht D, Lieberzeit PA. Raman and scanning probe microscopy for differentiating surface imprints of E. coli and B. cereus. *J Mater Chem B*. 2022;10(35):6758-6767. doi:http://doi.org/10.1039/D2TB00283C
33. Yang Y, Li G, Wang P, Fan L, Shi Y. Highly sensitive multiplex detection of foodborne pathogens using a SERS immunosensor combined with novel covalent organic frameworks based biologic interference-free Raman tags. *Talanta*. 2022;243:123369.
34. Tu Z, Cheng S, Dong H, et al. Universal and ultrasensitive detection of foodborne bacteria on a lateral flow assay strip by using wheat germ agglutinin-modified magnetic SERS nanotags. *RSC Adv*. 2022;12(42):27344-27354.
35. Huang X, Zhang Z, Chen L, et al. Multifunctional au nano-bridged nanogap probes as ICP-MS/SERS dual-signal tags and signal amplifiers for bacteria discriminating, quantitative detecting and photothermal bactericidal activity. *Biosens Bioelectron*. 2022:114414.
36. Rahman A, Kang S, Wang W, Huang Q, Kim I, Vikesland PJ. Lectin-modified bacterial cellulose nanocrystals decorated with au nanoparticles for selective detection of bacteria using surface-enhanced Raman scattering coupled with machine learning. *ACS Applied Nano Mater*. 2022;5(1):259-268.
37. Deb M, Hunter R, Taha M, Abdelbary H, Anis H. Rapid detection of bacteria using gold nanoparticles in SERS with three different capping agents: Thioglucose, polyvinylpyrrolidone, and citrate. *Spectrochim Acta Part A*. 2022;280:121533.
38. Ma X, Xu S, Li L, Wang Z. A novel SERS method for the detection of staphylococcus aureus without immobilization based on au@ ag NPs/slide substrate. *Spectrochim Acta Part A*. 2023;284:121757.
39. Xu Y, He P, Ahmad W, et al. Catalytic hairpin activated gold-magnetic/gold-core-silver-shell rapid self-assembly for ultrasensitive staphylococcus aureus sensing via PDMS-based SERS platform. *Biosens Bioelectron*. 2022;209:114240.
40. Juang R, Chen W, Cheng Y, et al. Fabrication of in situ magnetic capturing and Raman enhancing nanoplatelets for detection of bacteria and biomolecules. *Colloids Surf Physicochem Eng Aspects*. 2022;648:129189.
41. Shen W, Wang C, Zheng S, et al. Ultrasensitive multichannel immunochromatographic assay for rapid detection of foodborne bacteria based on two-dimensional film-like SERS labels. *J Hazard Mater*. 2022;437:129347.
42. Kim S, Ansah IB, Park JS, et al. Early and direct detection of bacterial signaling molecules through one-pot au electrodeposition onto paper-based 3D SERS substrates. *Sens Actuators B Chem*. 2022;358:131504.
43. Wang C, Wang C, Li J, Tu Z, Gu B, Wang S. Ultrasensitive and multiplex detection of four pathogenic bacteria on a bi-channel lateral flow immunoassay strip with three-dimensional membrane-like SERS nanostickers. *Biosens Bioelectron*. 2022;214:114525.
44. Xie B, Wang Z, Zhang R, Zhang Z, He Y. A SERS aptasensor based on porous au-NC nanoballoons for staphylococcus aureus detection. *Anal Chim Acta*. 2022;1190:339175.
45. Zhou S, Guo X, Huang H, et al. Triple-function Au–Ag-stuffed nanopancakes for SERS detection, discrimination, and inactivation of multiple bacteria. *Anal Chem*. 2022;94(15):5785-5796.
46. Li J, Khalenkow D, Volodkin D, Lapanje A, Skirtach AG, Parakhonskiy BV. Surface enhanced Raman scattering (SERS)-active bacterial detection by layer-by-layer (LbL) assembly all-nanoparticle microcapsules. *Colloids Surf Physicochem Eng Aspects*. 2022;650:129547.
47. Chen Y, Wang C, Chang W, et al. Hydrophilic–Hydrophobic nanohybrids of AuNP-immobilized two-dimensional nanomica platelets as flexible substrates for high-efficiency and high-selectivity surface-enhanced Raman scattering microbe detection. *ACS Applied Bio Mater*. 2022;5(3):1073-1083.
48. Qi X, Ye Y, Wang H, et al. An ultrasensitive and dual-recognition SERS biosensor based on Fe3O4@ au-teicoplanin and aptamer functionalized au@ ag nanoparticles for detection of staphylococcus aureus. *Talanta*. 2022:123648.
49. Chen Q, Zhao L, Liu H, et al. Nanoporous silver nanorods as surface-enhanced Raman scattering substrates. *Biosens Bioelectron*. 2022;202:114004. https://www.sciencedirect.com/science/article/pii/S0956566322000446. https://doi.org/10.1016/j.bios.2022.114004
50. Zhao H, Zheng D, Wang H, et al. In situ collection and rapid detection of pathogenic bacteria using a flexible SERS platform combined with a portable Raman spectrometer. *Int J Mol Sci*. 2022;23(13). https://doi.org/10.3390/ijms23137340
51. Jia N, Xiong Y, Wang Y, et al. A novel surface-enhanced Raman scattering method for detecting fish pathogenic bacteria with Fe3O4@PEI nanocomposite and concentrated Au@Ag. *J Raman Spectrosc*. 2022;53(2):211-221. https://doi.org/10.1002/jrs.6282
52. Zhu Y, Liu S, Li M, et al. Preparation of an AgNPs@Polydimethylsiloxane (PDMS) multi-hole filter membrane chip for the rapid identification of food-borne pathogens by surface-enhanced Raman spectroscopy. *Spectrochim Acta Part A*. 2022;267:120456. https://www.sciencedirect.com/science/article/pii/S1386142521010337. https://doi.org/10.1016/j.saa.2021.120456
53. Yang Y, Zeng C, Huang J, et al. Specific and quantitative detection of bacteria based on surface cell imprinted SERS mapping platform. *Biosens Bioelectron*. 2022;215:114524. https://www.sciencedirect.com/science/article/pii/S0956566322005644. https://doi.org/10.1016/j.bios.2022.114524
54. Wei W, Haruna SA, Zhao Y, Li H, Chen Q. Surface-enhanced Raman scattering biosensor-based sandwich-type for facile and sensitive detection of staphylococcus aureus. *Sens Actuators B Chem*. 2022;364:131929. https://www.sciencedirect.com/science/article/pii/S0925400522005718. https://doi.org/10.1016/j.snb.2022.131929
55. Wang Y, Ni H, Li H, Chen J, Zhang D, Fu L. Plasmonic microneedle arrays for rapid extraction, SERS detection, and inactivation of bacteria. *Chem Eng J*. 2022;442:136140. https://www.sciencedirect.com/science/article/pii/S1385894722016382. https://doi.org/10.1016/j.cej.2022.136140
56. Asgari S, Dhital R, Aghvami SA, Mustapha A, Zhang Y, Lin M. Separation and detection of E. coli O157:H7 using a SERS-based microfluidic immunosensor. *Microchim Acta*. 2022;189(3):111. https://doi.org/10.1007/s00604-022-05187-8
57. Chen K, Lee S, Kok L, Ishdorj T, Chang H, Tseng F. A 3D-ACEK/SERS system for highly efficient and selectable electrokinetic bacteria concentration/detection/antibiotic-susceptibility-test on whole blood. *Biosens Bioelectron*. 2022;197:113740. https://www.sciencedirect.com/science/article/pii/S0956566321007776. https://doi.org/10.1016/j.bios.2021.113740
58. Zhuang J, Zhao Z, Lian K, et al. SERS-based CRISPR/cas assay on microfluidic paper analytical devices for supersensitive detection of pathogenic bacteria in foods. *Biosens Bioelectron*. 2022;207:114167. https://www.sciencedirect.com/science/article/pii/S095656632200207X. https://doi.org/10.1016/j.bios.2022.114167
59. Zhao W, Zhang D, Zhou T, et al. Aptamer-conjugated magnetic Fe3O4@Au core-shell multifunctional nanoprobe: A three-in-one aptasensor for selective capture, sensitive SERS detection and efficient near-infrared light triggered photothermal therapy of staphylo-

coccus aureus. *Sens Actuators B Chem*. 2022;350:130879. https://www.sciencedirect.com/science/article/pii/S0925400521014477. https://doi.org/10.1016/j.snb.2021.130879
60. Pan L, Zhang P, Daengngam C, Peng S, Chongcheawchamnan M. A review of artificial intelligence methods combined with Raman spectroscopy to identify the composition of substances. *J Raman Spectrosc*. 2022;53(1):6-19. https://doi.org/10.1002/jrs.6225
61. Liu W, Wei L, Wang D, et al. Phenotyping bacteria through a black-box approach: Amplifying surface-enhanced Raman spectroscopy spectral differences among bacteria by inputting appropriate environmental stress. *Anal Chem*. 2022;94(18):6791-6798. https://doi.org/10.1021/acs.analchem.2c00502
62. Arslan AH, Ciloglu FU, Yilmaz U, Simsek E, Aydin O. Discrimination of waterborne pathogens, cryptosporidium parvum oocysts and bacteria using surface-enhanced Raman spectroscopy coupled with principal component analysis and hierarchical clustering. *Spectrochim Acta Part A*. 2022;267:120475. https://www.sciencedirect.com/science/article/pii/S1386142521010520. https://doi.org/10.1016/j.saa.2021.120475
63. Shakeel M, Majeed MI, Nawaz H, et al. Surface-enhanced Raman spectroscopy for the characterization of pellets of biofilm forming bacterial strains of staphylococcus epidermidis. *Photodiagn Photodyn Ther*. 2022;40:103145. https://www.sciencedirect.com/science/article/pii/S1572100022004318. https://doi.org/10.1016/j.pdpdt.2022.103145
64. Vaitiekūnaitė D, Bružaitė I, Snitka V. Endophytes from blueberry (vaccinium sp.) fruit: Characterization of yeast and bacteria via label-free surface-enhanced Raman spectroscopy (SERS). *Spectrochim Acta, Part A*. 2022;275:121158. https://www.sciencedirect.com/science/article/pii/S1386142522003079. https://doi.org/10.1016/j.saa.2022.121158
65. Grosso RA, Walther AR, Brunbech E, et al. Detection of low numbers of bacterial cells in pharmaceutical drug product using Raman spectroscopy and PLS-DA multivariate analysis. *BioRxiv*. 2022;2022:489535. http://biorxiv.org/content/early/2022/04/26/2022.04.26.489535.abstract. https://doi.org/10.1101/2022.04.26.489535
66. Zhu A, Ali S, Xu Y, Ouyang Q, Wang Z, Chen Q. SERS-based Au@Ag NPs solid-phase substrate combined with chemometrics for rapid discrimination of multiple foodborne pathogens. *Spectrochim Acta Part A*. 2022;270:120814. https://www.sciencedirect.com/science/article/pii/S1386142521013913. 10.1016/j.saa.2021.120814
67. Rebrošová K, Bernatová S, Šiler M, et al. Raman spectroscopy—a tool for rapid differentiation among microbes causing urinary tract infections. *Anal Chim Acta*. 2022;1191:339292. https://www.sciencedirect.com/science/article/pii/S0003267021011181. https://doi.org/10.1016/j.aca.2021.339292
68. Tang J, Li J, Yin X, et al. Rapid discrimination of clinically important pathogens through machine learning analysis of surface enhanced Raman spectra. *Front Microbiol*. 2022;13. https://www.frontiersin.org/articles/10.3389/fmicb.2022.843417
69. Zhang J, Zhang J, Ding J, Lin Q, Young GM, Jiang C. Rapid identification of live and dead salmonella by surface-enhanced Raman spectroscopy combined with convolutional neural network. *Vib Spectrosc*. 2022;118:103332. https://www.sciencedirect.com/science/article/pii/S0924203121001272. https://doi.org/10.1016/j.vibspec.2021.103332
70. Sun J, Xu X, Feng S, et al. Rapid identification of salmonella serovars by using Raman spectroscopy and machine learning algorithm. *Talanta*. 2023;253:123807. https://www.sciencedirect.com/science/article/pii/S0039914022006038. https://doi.org/10.1016/j.talanta.2022.123807
71. Zhang J, Liu Y, Li H, et al. Discrimination of periodontal pathogens using Raman spectroscopy combined with machine learning algorithms. *J Innov Opt Health Sci*. 2022;15(03):2240001. https://doi.org/10.1142/S1793545822400016
72. Tewes TJ, Kerst M, Platte F, Bockmühl DP. Raman microscopic identification of microorganisms on metal surfaces via support vector machines. *Microorganisms*. 2022;10(3). https://doi.org/10.3390/microorganisms10030556
73. Zahn J, Germond A, Lundgren AY, Cicerone MT. Discriminating cell line specific features of antibiotic-resistant strains of escherichia coli from Raman spectra via machine learning analysis. *J Biophotonics*. 2022;15(7):e202100274. https://doi.org/10.1002/jbio.202100274
74. Liu S, Zhu Y, Li M, et al. Rapid identification of different pathogenic spore-forming bacteria in spice powders using surface-enhanced Raman spectroscopy and chemometrics. *Food Anal Methods*. 2022;15(10):2810-2820. https://doi.org/10.1007/s12161-022-02326-y
75. Du Y, Han D, Liu S, et al. Raman spectroscopy-based adversarial network combined with SVM for detection of foodborne pathogenic bacteria. *Talanta*. 2022;237:122901. https://www.sciencedirect.com/science/article/pii/S0039914021008237. https://doi.org/10.1016/j.talanta.2021.122901
76. Rho E, Kim M, Cho SH, et al. Separation-free bacterial identification in arbitrary media via deep neural network-based SERS analysis. *Biosens Bioelectron*. 2022;202:113991. https://www.sciencedirect.com/science/article/pii/S0956566322000318. https://doi.org/10.1016/j.bios.2022.113991
77. Zhu C, Liu W, Wang D, Gong Z, Fan M. Boosting bacteria differentiation efficiency with multidimensional surface-enhanced Raman scattering: The example of bacillus cereus. *Luminescence*. 2022;37(7):1145-1151. https://doi.org/10.1002/bio.4268
78. Tian T, Yi J, Liu Y, et al. Self-assembled plasmonic nanoarrays for enhanced bacterial identification and discrimination. *Biosens Bioelectron*. 2022;197:113778. https://www.sciencedirect.com/science/article/pii/S0956566321008150. https://doi.org/10.1016/j.bios.2021.113778
79. Zhu Y, Liu W, Liu S, et al. Preparation of AgNPs self-assembled solid-phase substrate via seed-mediated growth for rapid identification of different bacterial spores based on SERS. *Food Res Int*. 2022;160:111426. https://www.sciencedirect.com/science/article/pii/S0963996922004835. https://doi.org/10.1016/j.foodres.2022.111426
80. Zhang W, He S, Hong W, Wang P. A review of Raman-based technologies for bacterial identification and antimicrobial susceptibility testing. *Photonics*. 2022;9(3). https://doi.org/10.3390/photonics9030133
81. Mushtaq A, Nawaz H, Irfan Majeed M, et al. Surface-enhanced Raman spectroscopy (SERS) for monitoring colistin-resistant and susceptible E. coli strains. *Spectrochim Acta, Part A*. 2022;278:121315. https://www.sciencedirect.com/science/article/pii/S1386142522004644. https://doi.org/10.1016/j.saa.2022.121315
82. Ditta A, Majeed MI, Nawaz H, et al. Surface-enhanced Raman spectral investigation of antibacterial activity of zinc 3-chlorobenzoic acid complexes against gram-positive and gram-negative bacteria. *Photodiagn Photodyn Ther*. 2022;39:102941. https://www.sciencedirect.com/science/article/pii/S1572100022002277. https://doi.org/10.1016/j.pdpdt.2022.102941
83. Gukowsky JC, Yang T, He L. Assessment of three SERS approaches for studying E. coli O157:H7 susceptibility to ampicillin. *Spectrochim Acta Part A*. 2022;264:120239. https://www.sciencedirect.com/science/article/pii/S1386142521008167. https://doi.org/10.1016/j.saa.2021.120239
84. Zhang P, Wu X, Su L, et al. Rapid, label-free prediction of antibiotic resistance in salmonella typhimurium by surface-enhanced Raman spectroscopy. *Int J Mol Sci*. 2022;23(3). https://doi.org/10.3390/ijms23031356
85. Gukowsky JC, He L. Development of a portable SERS method for testing the antibiotic sensitivity of foodborne bacteria. *J Microbiol Methods*. 2022;198:106496. https://www.

sciencedirect.com/science/article/pii/S0167701222000914. https://doi.org/10.1016/j.mimet.2022.106496

86. Chen J, Yang J, Chen W, et al. Tri-functional SERS nanoplatform with tunable plasmonic property for synergistic antibacterial activity and antibacterial process monitoring. *J Colloid Interface Sci*. 2022;608:2266-2277. https://www.sciencedirect.com/science/article/pii/S0021979721018099. https://doi.org/10.1016/j.jcis.2021.10.132
87. Wei L, Tang Jia-Wei, Lyu Jing-Wen, et al. Discrimination between carbapenem-resistant and carbapenem-sensitive klebsiella pneumoniae strains through computational analysis of surface-enhanced Raman spectra: A pilot study. *Microbiol Spectrum*. 2022;10(1):2409. https://doi.org/10.1128/spectrum.02409-21
88. Zhang P, Fu Y, Zhao H, et al. Dynamic insights into increasing antibiotic resistance in staphylococcus aureus by label-free SERS using a portable Raman spectrometer. *Spectrochim Acta Part A*. 2022;273:121070. https://www.sciencedirect.com/science/article/pii/S1386142522002189. https://doi.org/10.1016/j.saa.2022.121070
89. Saleem M, Majeed MI, Nawaz H, et al. Surface-enhanced Raman spectroscopy for the characterization of the antibacterial properties of imidazole derivatives against bacillus subtilis with principal component analysis and partial least Squares–Discriminant analysis. *Anal Lett*. 2022;55(13):2132-2146. https://doi.org/10.1080/00032719.2022.2047997
90. Nakar A, Pistiki A, Ryabchykov O, Bocklitz T, Rösch P, Popp J. Detection of multi-resistant clinical strains of E. coli with Raman spectroscopy. *Anal BioanalChem*. 2022;414(4):1481-1492. https://doi.org/10.1007/s00216-021-03800-y
91. Lu J, Chen J, Liu C, et al. Identification of antibiotic resistance and virulence-encoding factors in klebsiella pneumoniae by Raman spectroscopy and deep learning. *Microb Biotechnol*. 2022;15(4):1270-1280. https://doi.org/10.1111/1751-7915.13960
92. Lin S, Chao P, Cheng H, et al. An antibiotic concentration gradient microfluidic device integrating surface-enhanced Raman spectroscopy for multiplex antimicrobial susceptibility testing. *Lab Chip*. 2022;22(9):1805-1814. doi:http://doi.org/10.1039/D2LC00012A
93. Olaniyi OO, Li H, Zhu Y, Cui L. Metabolic responses of indigenous bacteria in chicken faeces and maggots to multiple antibiotics via heavy water labeled single-cell Raman spectroscopy. *J Environ Sci*. 2022;113:394-402. https://www.sciencedirect.com/science/article/pii/S1001074221002527. https://doi.org/10.1016/j.jes.2021.06.024
94. Sun B, Kang X, Yue S, et al. A rapid procedure for bacterial identification and antimicrobial susceptibility testing directly from positive blood cultures. *Analyst*. 2022;147(1):147-154. doi:http://doi.org/10.1039/D1AN01210J
95. Zhang W, Chen X, Zhang J, et al. Rapid antimicrobial susceptibility testing for mixed bacterial infection in urine by AI-stimulated Raman scattering metabolic imaging. *Med Novel Technol Devices*. 2022;16:100132. https://www.sciencedirect.com/science/article/pii/S2590093522000194. https://doi.org/10.1016/j.medntd.2022.100132
96. Yuan S, Chen Y, Lin K, et al. Single cell Raman spectroscopy deuterium isotope probing for rapid antimicrobial susceptibility test of elizabethkingia spp. *Front Microbiol*. 2022;13. https://www.frontiersin.org/articles/10.3389/fmicb.2022.876925
97. Klein D, Breuch R, Reinmüller J, Engelhard C, Kaul P. Discrimination of stressed and non-stressed food-related bacteria using Raman-microspectroscopy. *Foods*. 2022;11(10). https://doi.org/10.3390/foods11101506
98. Wang W, Rahman A, Huang Q, Vikesland PJ. Surface-enhanced Raman spectroscopy enabled evaluation of bacterial inactivation. *Water Res*. 2022;220:118668. https://www.sciencedirect.com/science/article/pii/S0043135422006212. https://doi.org/10.1016/j.watres.2022.118668
99. Bandeliuk O, Assaf A, Bittel M, Durand M, Thouand G. Development and automation of a bacterial biosensor to the targeting of the pollutants toxic effects by portable Raman spectrometer. *Sensors*. 2022;22(12). https://doi.org/10.3390/s22124352

How to cite this article: Rodriguez L, Zhang Z, Wang D. Recent advances of Raman spectroscopy for the analysis of bacteria. *Anal Sci Adv*. 2023;4:81–95. https://doi.org/10.1002/ansa.202200066