AITA
ARTIFICIAL INTELLIGENCE
THEORY AND APPLICATIONS

■ REVIEW ARTICLE

Artificial Intelligence, Microbiology, and Raman Technologies

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RECEIVED JULY 7, 2022
ACCEPTED SEPTEMBER 2, 2022

CITATION Özyaman, F., Yılmaz, Ö., (2022). Artificial Intelligence, Microbiology, and Raman Technologies. *Artificial Intelligence Theory and Applications, 2*(2), 59-68.

Abstract

Artificial intelligence which became important in the laboratory is used in medical microbiology in infectious disease testing to support decision-making, identification and antimicrobial susceptibility testing with Raman technologies, image analysis, and MALDI-TOF-MS. Antimicrobial resistance is a worldwide risk for human health. Treatment of infections requires fast and correct identification and antimicrobial susceptibility testing. Current microbiology laboratory procedures give broad information in identification and antimicrobial susceptibility testing; however, they are complex and time-consuming. Thus, new methods are required such as Raman technologies. Vibrational spectroscopy method Raman spectroscopy is one of the useful and new tools that is used in different fields of medicine. Recently, fast and accurate Raman technologies used identification, differentiation of resistant and sensitive strains, and antimicrobial susceptibility testing became important in microbiology. Raman technologies include various kinds of methods. Raman spectroscopy can implement identification, and antibiotic susceptibility together with increased accuracy. It is a cheap, label-free, and effective method that differentiates bacterial infections. Besides bacteria, it is also used in rapid and sensitive virus detection such as COVID-19 by using saliva. When PCR is used in COVID-19 detection, as the variants increase sensitivity decreases. Raman technology overcomes this problem. This review summarizes the applications, challenges, and future of Raman technologies in microbiology to improve the treatment of infectious diseases and improve human health.

Keywords: artificial intelligence, Raman technologies, identification, antimicrobial susceptibility testing

1. Introduction

Artificial intelligence (AI) related to computer programs solves problems and makes predictions like the human brain. Health data aided with AI help to improve our lives and is informative about the different characteristics of infections [1]. AI assists us in the efficiency, accuracy, and processing of large amounts of data in clinical microbiology to enhance health care. AI is used as expert rules in automated susceptibility tests and identification [2].

The worldwide increase in antimicrobial resistance (AMR) is a vital problem that seriously puts human health at risk [3-5]. Conventional microbiological techniques are long and generally, identification takes a day of incubation and antimicrobial susceptibility testing (AST) takes one to two more days [5]. To fight against the increase in AMR, fast

Artificial Intelligence Theory and Applications, ISSN: 2757-9778. ISBN: 978-605-69730-2-4 © 2022 University of Bakırçay

identification and AST are necessary. Recently, MALDI-TOF-MS is used for microbial detection and classification in medical microbiology [6]. After the identification of the microorganism, extra 18 to 24 hours are required for doing AST [6,7]. In conventional techniques, it takes three to five days including several days for getting pure culture and extra 20 hours for minimal inhibitory concentration (MIC). AST time is decreased to 4.5–18 h by automation but it still needs culture which is time-consuming [8]. Molecular methods are important in identification because of their raised specificity and sensitivity. But it is hard to implement in unknown microorganisms [9]. Raman technologies that have a significant role in the identification and AST [13-21] are label-free, non-invasive, and have single-bacterial sensitivity [10-12]. In this review, we highlight developments in Raman technologies related to rapid identification and AST [22-24].

2. Implementations of AI in Medical Microbiology

AI uses algorithms and helps in making decisions. In clinical microbiology, AI helps us to be more productive, and precise, bring out conclusions from data and can be applied to make health care better. AI is already used as expert rules in some automated susceptibility tests and identification in the laboratory [25].

AI supports medical microbiology in analyzing rich data such as images, spectra, and DNA-RNA sequences. Using ML in these data results in the detection of different properties of microorganisms. If AI is not used, analysis of rich data is manual, time-consuming, and needs experience. Taking into consideration the staff shortages and complicated analyses, AI supports clinical microbiology [26].

2.1. Gram Stain

AI application for automated interpretation of blood culture Gram stains was explained by Smith, Kang, and Kirby [27]. The convolutional neural network (CNN) was very good at image grouping. They used a pre-trained CNN which was trained to recognize Gram stain bacteria with different shapes from positive blood culture. Image crops given to the CNN were trained on a computer and crop classification accuracy was 95% and slide classification accuracy was 92.5%. However, with a high-powered computer, crop accuracy was >99% [27].

2.2. Culture Plate Images

Inoculation automating, interpretation automating by computer vision, and the total automation (including inoculation, incubation, and image taking) improve the digital plate reading of cultures [28). The combination of AI and chromogenic agar is as sensitive as nucleic acid amplification tests [30], and it is good at colony counts and discriminating colonies in urine cultures with 99.8% sensitivity, 68.5% specificity, and result obtaining time was decreased (4 h 42 min in negative samples, 3h 28 min in positive samples) [29].

Advanced AI defines properties of culture such as colony shape-count, and purity. Sensitivity and specificity were 99.8% and 72.0%, respectively (32).

2.3 MALDI-TOF MS

MALDI-TOF MS's advances such as identification directly from the sample and detection of antimicrobial resistance are applied and reported in studies [33].

Data analysis steps are pre-processing (decreases random noise, amplifies the true signal, and standardizes spectra), feature selection (algorithms that change spectrum to peak list), and classification (ML interprets spectra features) [34]. To differentiate *Staphylococcus aureus* strains' resistance to vancomycin, ML is used in data analysis [35].

The challenges are not being able to detect molecules over the m/z 2000-12000 [37], specimen processing and spectral techniques influencing spectra, spectra files, and the lack of spectral libraries and alternatives for quick AST and identification [38].

2.4. Whole Genome Sequencing

AI and ML are used in whole-genome sequencing (WGS) data to predict antimicrobial resistance. ML analyzes short DNA sequences and is used to study the correlation between their DNA sequence and their antimicrobial resistance. WGS data is used to predict antimicrobial resistance (S/I/R or MIC) minimum inhibitory concentration [39,40].

3. Identification and Raman Technologies

3.1. Raman Spectroscopy and AI

The vibrational spectroscopy method, Raman spectroscopy is used in medicine [40,10,44,45]. It is not like other approaches due to its uncomplicated usage, being label-free, and non-invasive. Besides these properties, it has high output and large-scale information about the structure and interactivities of bacterial molecules [46].

Raman spectroscopy identifies microorganism's species. Identification of bacteria in urine specimens was done directly by Raman microscopy and a statistical model without the need for culture [47]. In another study pathogenic staphylococcal strains were identified directly from colonies [48].

Challenge in identification is due to the weak Raman signals from bacteria. Presently, deep learning (DL) techniques are used to process the large data of Raman spectroscopy. Raman spectroscopy and DL are used in the identification of strains. The technique's advantage is that there is no need for culture, and data analysis time is decreased. The model's accuracy is 89.1% in the identification of methicillin-resistant and sensitive *S. aureus* [42].

The processing time of large data in DL models is long and needs powerful computers [42]. To solve the problem, a combination of Raman spectroscopy and ML is used for fast identification of bacterial strains at the serotype level and accuracy is 87.1% - 95.8%. ML 's data interpretation is easier than DL and ML. ML does not need powerful computers, so integration of ML to portable Raman spectrometers makes it simpler to broaden to the clinic [49].

The disadvantage of this method is the weak signal and degradation of the specimen due to the powerful laser. [50]. In COVID-19 detection, PCR sensitivity decreases as variants increase since reagents are less specific. In the reagent-free technique for COVID-19, ML can be updated according to variants without requiring new reagents. A non-invasive, label-free Raman spectroscopy and ML combination is used to detect changes in saliva and differentiated between COVID-positive and COVID-negatives [85].

3.2. Surface-Enhanced Raman Spectroscopy (SERS)

SERS analyses varying specimens quickly and increases the sensitivity. By using the interactivity roughness with molecules in the specimen, SERS provides signal enhancement. As substrates, gold-silver nanoparticles are used [38]. Due to Raman microscopy being a potent instrument in identification, SERS and aptamer-based SERS are also used to identify foodborne pathogens [51-53].

In the tigecycline-resistant *E. coli* identification study, resistant and sensitive strains are experimented by using statistical analysis and SERS and concluded that using the combination of the analysis model and SERS, enables differentiating the species [54]. The method will be improved if the problems related to the procedures, spectrum reproducibility, substrate stability, and setting up of a SERS database are solved [13].

In the SERS sensor study, the SERS sensor can identify Gram (-) and Gram (+) pathogens, needs a very small sample, is economic, accurate, and takes less than 5 minutes [16]. In another study, 20 bacterial strains are identified quickly, sensitively, and specifically with a SERS sensor by using a silver nanorod substrate to get the bacteria fingerprint. The method is easy and economical [55]. The combination of microfluidics and SERS applied in the user-friendly microfluidic instrument allows the identification of pathogens in clinical microbiology in 15 minutes [56].

By improving the challenges (e.g. improving the spectra and output, analysis optimization, and decreasing the price of the substrate), SERS allows real-time monitoring and identifies bacteria quickly [41,50]. In the detection of SARS-CoV-2, label-free surface-enhanced Raman Scattering with silver nanoparticles to get the fingerprints is used [86].

3.3. Laser Tweezers Raman Spectroscopy (LTRS)

The technique identifies the microorganism without destruction of the cells. Raman spectroscopy images give information about RNA/DNA, lipids, and proteins in specimens. Advantages are easy specimen procedure, keeping the specimens in original condition, using minimum specimens, and being very sensitive and specific [57,58]. In reagent-free identification by confocal LTRS, diverse bacteria are differentiated at aqueous conditions quickly [59].

For more precise identification by DL, DL is used for LTRS spectra analysis at the single-cell level. Spectral signatures from bacteria were obtained by CNN and accuracy was 95.64 %. The study revealed that LTRS was important in the identification of unculturable bacteria [60]. For improvement of the method, automated and small instruments, various bacterial spectra advancement, and fast data systems are required.

3.4. Coherent anti-Stokes Raman Scattering Spectroscopy (CARS)

CARS microscopy method is label-free, and it has increased signals, output, and specificity [61]. The hyperspectral CARS method gives information about the image very precisely and identifies at the single-cell level, in minutes, in complex specimens (urine) without culture and labelling [62]. Anthrax endospores are identified without any destruction by this method [63].

Due to the simplicity and cost-effectiveness of the method, the CARS microscope can be a mobile and economic tool in clinical diagnosis. DL is combined with the method for

identification and obtaining bacteria's metabolic feedback to antibiotics by using spectrum changes, and DL helps in both identification and AST [64].

4. AST and Raman Technologies

4.1. Raman Spectroscopy

Raman spectroscopy gives microbial molecules' fingerprint information [65] and signal intensity is related to these molecules' concentration [16,66]. It identifies the changes that show bacteria's feedback to antibiotics in bacterial molecules [19,67]. The method can be used in complex specimens in clinics with a low number of bacteria since Raman can identify single bacteria without culture [68,69].

In the study of AST of MRSA by single-cell Raman, susceptible and resistant strains' spectra changed according to the cefoxitin concentrations, and resistance/sensitive strains were found in large bacterial populations [71]. In AST by single-cell Raman and tweezers combination method, *E. coli'* s feedback to penicillin-cefazolin and their interactivity were studied [72]. In another study, *E. coli* strains' antibiotic resistance genes were shown by the method [70]. Above mentioned techniques show known resistance and they do not show unknown resistance.

4.2. Raman Spectroscopy and Isotope Labelling

AST by Raman spectroscopy and isotope labelling combination evaluates the bacterial metabolism degree. When bacteria take in isotopic markers, compounds in the bacteria show a redshift in spectra [16]. Consequently, the method is efficient in microbial activity studies. Deuterium (D_2O) labelling is very sensitive in microbial metabolism studies. It is used in the identification and microbial activity in medical and environmental specimens [73].

Raman-D_2O combination focuses on the microbial metabolic activity and bacteria-antibiotic interaction [19] and is used for fast AST in urine specimens and results are obtained in 2.5 h [67]. Linear discriminant analysis (LDA) Gram stain grouping and D_2O were combined to form fast Raman-assisted AST (FRAST) which shows Gram grouping and microbial metabolic activity in urinary specimens. In urine, AST resulted in 3h. The limitation is defining only the sensitivity, not the MIC [74].

4.3. SERS Sensors

SERS sensors are used in AST, biomarkers, DNA, RNA, and quantitation [75]. SERS spectra is analyzed with ML, and DL to support doctors for precise evaluations in AST [76].

Bacteria-aptamer@AgNPs-SERS combination identifies *E. coli* and *S. aureus'* Raman intensity to the antibiotics and their MIC is determined in 1 h [77]. In another SERS sensors study, ML analyzes the data and shows metabolic degree after the antimicrobial application. Method differentiates *E. coli* and *P. aeruginosa* reactions with 99% accuracy in 10 min. Additionally DL analyzed the spectra when *P. aeruginosa* was treated with antibiotics and showed the difference between resistant, sensitive, and untreated spectra [78].

4.4. SERS and Microfluidics

Microfluidics improves SERS's efficiency in complex specimens [79] and controls fluid activity and combines procedures in portable instruments to have increased output and fast results with minimum specimens [80].

Microfluidics combining membrane filtration and SERS (MF-SERS) is developed for AST. The MF-SERS decreases the microbial culture time, contamination, and error. It is small and integrates culture, and AST [81]. Limitations are the requirement of complicated pre-treatment for specimens and being expensive.

4.5. SRS and Deuterium Labelling

CRS microscopy has signal improvements and is used in label-free imaging, medicine follow-up, cancer detection, quantitation, metabolism, and enzymes [82] and it has rapid imaging and high resolution. The method studies microbial metabolic activity at the single-bacteria level [83].

When the technique is combined with MALDI-TOF MS, pathogens are identified, and AST results are obtained in 3.5 h from (+) blood cultures directly [85].

The method is rapid, direct on specimens, and measures all bacteria in the field but is complicated and expensive.

5. Raman Technologies and Medical Microbiology

In spite of huge improvements in medical science over the past decades, precise and quick bacterial identification and their virulence factors such as AMR and biofilms still have problems. Timely identification of pathogens is necessary to select appropriate antibiotic treatment and correct care for patients. This decreases hospital costs, therapy period, and the growth of AMR, and saves the lives of humans [88]. The present gold standard is the cultivation of bacteria and identification based on their morphological and metabolic features. Although this method is traditional and reliable, critical drawbacks are that cultivation can take several days and a large number of bacteria are not culturable, consequently not accessible with this method. Therefore, in some situations, only bacteria that are in a viable but not culturable (VBNC) condition can be available [88].

The state-of-the-art of Raman in bacterial identification and to detect antimicrobial resistance is important in the diagnosis of infection. Compared to the conventional methods, Raman methods with the increased potential have many advantages such as differentiating the strains by spectral fingerprint rapidly and accurately. Besides, it is an early, economical, and easy identification of bacteria by direct examination of colonies on agar plates and AST together. It does not require any cultivation in some Raman methods to identify bacteria in human body fluids. It is non-destructive for the viability of bacteria and does not require labelling. It has improved algorithms, data analysis, and databases for sensitivity and specificity. This method allows single cell or molecule detection in various and in minimum samples (e.g. blood, urine) with SERS [87].

Raman spectroscopy is an effective optical technique and remarkably supports the quick diagnosis of diseases. It enables the identification of bacteria, virulence factors, AMR, and biofilm formation. Therefore, it has a great potential as a productive and practical solution for identification of pathogenic bacteria and AST [88].

6. Conclusions

Fast identification and AST decrease the AMR and save lives. Many scientists focused on different techniques for the improvement of Raman technologies in medicine. AI supports data analysis to obtain information from Raman spectra. Requirements are improving the understanding of spectra, reproducibility of spectral data and high-output detection, specimen preparation optimization; automation, and decreasing prices. Raman methods are shorter than the culture and help in decision-making. Raman technologies can be combined with other technologies and automation to be more effective. In order to develop Raman techniques, further research is required.

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