



ARTIFICIAL INTELLIGENCE-BASED TOOLS APPLIED TO PATHOLOGICAL DIAGNOSIS OF MICROBIOLOGICAL DISEASES

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Abstract

Pathological testing is an important part of the world diagnostic picture of infectious complications, which forms the necessary evidence in making clinical decisions in various healthcare facilities. Traditional microbiological methods, including microscopy, culture and molecular assays, are the practice standard, however, these modalities are often labour-intensive, time-consuming and depend on expert knowledge, which adds to extended diagnostic delays as well as systemic inefficiencies. Artificial intelligence in recent years has become a disruptive technology in laboratory automation through the implementation of Pathology-Digital Systems, which are driven by Digital Image Analysis. These tools are designed to support clinical practice by helping to identify pathogens using modern digital pathology and identifying the microbe using machine learning algorithms and interpreting the complex datasets of the molecules. Convolutional neural networks have already been shown to perform at the state-of-the-art level when used as diagnostic instruments to assess Gram-stained smears, peripheral blood preparations and histological sections and identify a large range of microorganisms- bacterial, viral, fungal and parasitic agents with high precision. AI and advanced forms of diagnostics like polymerase chain reaction (PCR), next-generation sequencing (NGS), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have

contributed ample to the detection of pathogens, anticipation of antimicrobial resistance, and outbreak surveillance. However, issues related to data standardisation, model interpretability, and regulatory implementation during the implementation of AI in diagnostic platforms remain, but AI promises to enhance the effectiveness, accuracy, and clinical decision-making skills of microbiologists and pathologists in the laboratory diagnosis of infectious diseases. This review presents the current advances, real-world applications, current constraints, and future trends of AI-based diagnostic devices in the pathological microbiology domain.

Key Words; Artificial Intelligence, Machine Learning, Deep Learning, Microbiological Diseases, Digital Pathology, Clinical Microbiology, Molecular Diagnostics

1. Introduction

The microbiological diseases continue to be among the main causes of morbidity and mortality globally, which explains the need to develop more fast and accurate diagnostic techniques [1]. Pathological diagnosis plays a central role of determining infectious agents and assisting in the provision of relevant therapeutic interventions. The standard microbiological diagnostic methods, which are microscopy, culture, and molecular laboratory tests, are stable but require significant resources in terms of time, labor, and expertise to perform. These limitations may trigger the occurrence of delays between diagnosis and treatment, especially in resource constrained and high-burden settings [2]. The development of artificial intelligence has become a new approach to improve the process of handling complex pathological data, by automated processing and information interpretation of data obtained by these classic diagnostic procedures. Through machine learning and deep learning algorithms, AI systems have the ability to analyze large volumes of data and draw conclusions through the results of the analysis, eliminating the need to involve as many humans as possible [3,4]. The microbiological diseases continue to be among the main causes of morbidity and mortality globally, which explains the need to develop more fast and accurate diagnostic techniques. Pathological diagnosis plays a central role of determining infectious agents and assisting in the provision of relevant therapeutic interventions [5]. The standard microbiological diagnostic methods, which are microscopy, culture, and molecular laboratory tests, are stable but require significant resources in terms of time, labor, and expertise to perform. These limitations may trigger the occurrence of delays between diagnosis and treatment, especially in resource constrained and high-burden settings. The development of artificial intelligence has become a new approach to improve the process of handling complex pathological data, by automated processing and information interpretation of data obtained by these classic diagnostic procedures. Through machine learning and deep learning algorithms, AI systems have the ability to analyze large volumes of data and draw conclusions through the results of the analysis, eliminating the need to involve as many humans as possible [6,7].

2. Burden and Challenges of Microbiological Diseases in Pathological Diagnosis

The microbiological diseases continue to be among the main causes of morbidity and mortality globally, which explains the need to develop more fast and accurate diagnostic techniques. Pathological diagnosis plays a central role of determining infectious agents and assisting in the provision of relevant therapeutic interventions [8]. The standard microbiological diagnostic methods, which are microscopy, culture, and molecular laboratory tests, are stable but require significant resources in terms of time, labor, and expertise to perform. These limitations may trigger the occurrence of delays between diagnosis and treatment, especially in resource constrained and high-burden settings [9]. The development of artificial intelligence has become a new approach to improve the process of handling complex pathological data, by automated processing and information interpretation of data obtained by these classic diagnostic procedures. Through machine learning and deep learning algorithms, AI systems have the ability to analyze large volumes of data and draw conclusions through the results of the analysis, eliminating the need to involve as many humans as possible. Most microbial pathogens are highly specific to grow, and it might take weeks or even

months to grow them; which further presents pathologists with diagnostic challenges [10]. The major problem in the diagnostic laboratories is that due to the large workload, there is an increase in the turnaround time and inconsistency of results across the observers. The person who interprets the stained smear or the histopathological section manually is prone to human error or exhaustion which may lead to inaccurate diagnosis. The shortage of resources and standardized practices, and the unavailability of advanced molecular technologies, are some of the reasons that lead to the many diagnostic dilemmas in such settings. The high rate of evolution of pathogenic organisms, the appearance of new infectious agents, requires the creation and introduction of diagnostic tools that are flexible, scalable, and can accept large amounts of complex data. There is, therefore, an urgent requirement of innovative diagnostic procedures that will help the pathologists and microbiologists deal with the growing burden of microbiological disease. High-technology tools and especially those based on AI are one of the possible ways to overcome the existing constraints on the quality and efficiency of pathological diagnosis [11].

3. Conventional Diagnostic Approaches and Their Limitations

The basis of a vast array of methodologies geared towards diagnosing biological defects in human beings and other animals due to microorganisms is traditional diagnostic models. Direct microscopy is one of the methods that includes examination of stained smear preparations of pathological tissues. Culture methods make it easier to isolate microorganisms and determine their antigenic profiles [12]. The biochemical identification measures the metabolic characteristics of microorganisms which include fermentation of sugars and the production of lactic acids. Serological identification tests are tests that identify the presence of antibodies/ antigens linked to viruses or parasites. The polymerase chain reaction (PCR) technology allows amplification of a single DNA sequence which allows the identification of specific microorganisms. There is a modality that is applicable and a range of techniques that can be used to make the right diagnosis about all types of infectious disease in a timely manner. Skimming Liability Unethical liability/Obligations In addition, the use of antibiotics in the past may reduce the percentage of positive cultures that are acquired on patients with a particular infection. The basis of a vast array of methodologies geared towards diagnosing biological defects in human beings and other animals due to microorganisms is traditional diagnostic models. Direct microscopy is one of the methods that includes examination of stained smear preparations of pathological tissues. Culture methods make it easier to isolate microorganisms and determine their antigenic profiles [13]. The biochemical identification measures the metabolic characteristics of microorganisms which include fermentation of sugars and the production of lactic acids. Serological identification tests are tests that identify the presence of antibodies/ antigens linked to viruses or parasites. The polymerase chain reaction (PCR) technology allows amplification of a single DNA sequence which allows the identification of specific microorganisms. There is a modality that is applicable and a range of techniques that can be used to make the right diagnosis about all types of infectious disease in a timely manner. Skimming Liability Unethical liability/Obligations In addition, the use of antibiotics in the past may reduce the percentage of positive cultures that are acquired on patients with a particular infection [14].

4. AI-Based Image Analysis and Digital Pathology in Microbiology

Image analysis technologies supported by artificial intelligence (AI) deployment is a crucial component of the field of digital pathology as it is implemented in microbiology. The use of AI-assisted image analysis can be used to automate the process and significantly increase the throughput of digital image assessment of microscopic and histopathological samples [15]. The introduction of computerized laboratory resources has made possible the use of advanced machine learning paradigms, including deep learning models, to detect and categorize microorganisms in a continuous visual field, e.g. a full slide, and therefore avoids the need to analyze samples in different positions. CNNs, which are part of this type of AI-based solutions, have been shown to have a higher level of expertise in identifying complicated visual patterns and complex morphological features that are relevant to pathogenic organisms [16]. Within the framework of clinical microbiology, AI-based

image analysis systems are utilized in the wide range of diagnostic sample types, such as Gram-stained smears, peripheral blood films, acid-fast bacilli (AFB) smears, fungal preparation slides, and histopathological sections. As a result, these solutions can easily and successfully detect bacterial, parasitic, and fungal morphologies and viral cytopathic changes, and only a small amount of manual validation of the resulting outputs is necessary. Digital Pathology Bryan Hall 12:59:31 World Health Organization World Health Organisation I do not believe so. Moreover, the rapid working conditions created by the introduction of artificial intelligence (AI) image-analysis have multiplied the time needed to conduct laboratory analyses, by the automatic processing of thousands of slides. Such automation reduces total workload of the laboratory, as well as, shortens the time of specimen analysis. Some of the studies that have been conducted today were using AI as a means to supplement and optimize the analysis of the specimens, such as the identification of *Mycobacterium tuberculosis* using ZiehlNeelsen staining, the detection of *Plasmodium malaria* parasites in peripheral blood films, and the differentiation of fungal and parasitic specimens based on the features of the microscopic images. These studies demonstrate the increased application of AI techniques in diagnostic pathology. The further use of AI systems in the analysis of tissue-biopsy that will help to differentiate between infectious and non-infectious inflammatory syndromes is currently being considered. The purpose of such efforts is to broaden the diagnostic scope of AI to the traditional cytology [17]. The digital pathology applications based on AI allow managing the data efficiently and effectively, provide an opportunity to consult with experts that are far away, and help to implement tele-pathology services. The latter capabilities are especially useful in the setting where the lack of resources in healthcare or geographic isolation interferes with access to specialist diagnostics. However, as much as these benefits are there, there are still a number of challenges. The variability in image quality, the heterogeneity of datasets and the high requirement of expert annotation of microscope slides still hinder the ubiquity of AI solutions. These problems highlight the need of a strict data curation and validation. Continued improvements in algorithm development, enhanced computing capabilities and the ever-increasing presence of curated data are creating additional opportunities to broaden the use of AI-based image analysis in the work of laboratory researchers in the microbiological pathology laboratory [18]. With increased application of laboratory automation and molecular diagnostics, the diagnosis of microbiological diseases is increasingly efficient and accurate with the help of artificial intelligence (AI) [26]. With the development of technology, information for microbiological disease keeps increasing rapidly, and molecular diagnostics technologies (e.g., multiplex PCR, real-time PCR, NGS, isothermal amplification) can generate complex data in large volume that needs to be analyzed by sophisticated analytic techniques such as ML (machine learning), DL (deep learning).

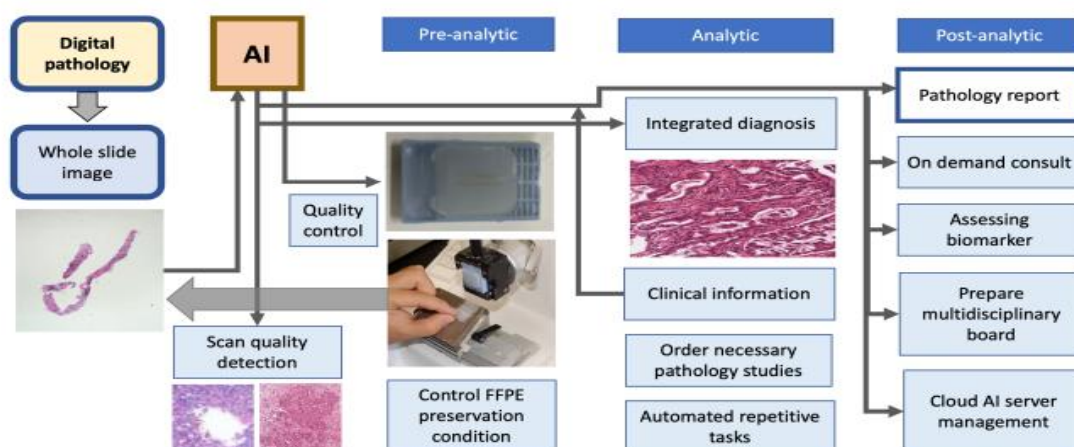


Figure 1 demonstrates that artificial intelligence and digital pathology can be used to improve the efficiency of workflow in a pathology laboratory. One such example involves providing whole-slide images, obtained using digital pathology equipment, to an AI system, which may offer control, verification, and guidance at all steps of the pre-analytical, analytical, and post-analytical pathology workflow [19].

5. Machine Learning and Deep Learning Applications for Microbial Identification

Over the past years, interest among scholars has shifted more towards automated and computer-based pattern recognition with the use of machine learning and deep learning algorithms to elevate microbial recognition by examining large, high-dimensional biological data sets [20]. Complementarily, support vector machines, random forests and k-nearest neighbours are machine learning algorithms, and convolutional and recurrent neural networks are deep learning architectures, which have been widely used to identify and classify organisms in the field of microbiology [21,22]. The models made using artificial intelligence have been shown to perform better and differentiate bacterial species using their phenotypic, metabolic, and growth traits. In combination with new technologies, such as mass spectrometry (MALDI-TOF), machine learning algorithms can enhance the quality of spectral measurements and provide accurate and timely species-level identifications of some fungi and bacteria. Another interesting implementation of deep learning in the sphere of genomic and proteomic data analysis is the ability to identify virulence factors, identify the type of strain, and predict susceptibility of microbial strains to antimicrobial agents [23]. Deep-learning algorithms are being used to improve the detection of multidrug-resistant organisms through molecular microbiology. The algorithms help the investigators to process the polymerase chain reaction (PCR) and next-generation sequencing (NGS) data, allowing one to distinguish among bona-fide pathogens and background noise, as well as to identify possible contaminants existing in the samples. Metagenomic analysis which is powered by artificial-intelligence enables nowadays the identification of a variety of pathogens in the polymicrobial setting even in the case when the operator is not aware of the etiological agents that are involved. In addition, machine-learning-based systems are increasingly becoming more diagnostic through enabling the computational model to self-learn based on large repositories of previous positive and negative test results, thus changing with emergent or changing pathogenic agents as well as with changing microbial communities. Even though the deep-learning algorithms could provide significant improvements in the speed and accuracy of comparison with the traditional diagnostic modalities, the eventual effectiveness of AI-based diagnostics (AIBD) will be dependent on the quality and diversity of the training data sets [24]. The adoption of machine-learning and deep-learning methods on microbial identification, in turn, it is a considerable technological innovation, which helps with precision microbiology in clinical practice, complementing but not replacing current diagnostic methods [25].

6. Integration of Artificial Intelligence with Molecular Diagnostics and Laboratory Automation

Microbiological disease diagnosis is becoming more efficient and accurate due to greater use of artificial intelligence (AI) with laboratory automation and molecular diagnostics [26]. As technology advances, the amount of available data on microbiological disease is increasing significantly, then molecular diagnostic approaches such as multiplex PCR, real-time PCR, next-generation sequencing (NGS), and isothermal intensification produce large amounts of complicated data that must be processed via advanced analytical methods such as machine learning and deep learning procedures. Mechanizing the processing of these datasets reduces both the time and risk of errors associated with manual processing of the data [27]. As per machine learning and deep learning models are applied to increase sensitivity and specificity for molecular diagnostics, AI-driven software tools assist users in developing primers and probes and optimizing PCR conditions, enabling the user to identify "true" positive signals rather than coincidental or background sound. In addition, AI will assist in alignment of nucleotide sequences for NGS-based diagnostics, organize specific pathogens and classify resistance genes and virulence factors. The diagnostic use of these applications is particularly relevant in classifying polymicrobial infections and detecting new pathogens, as well as monitoring their outbreaks in real time [28]. Integrated with artificial intelligence (AI), laboratory automation systems support to further streamline the process of diagnosing by providing a matched workflow (e.g., sample dispensation, data acquisition, result reporting) to facilitate completion. AI-enabled laboratory material systems are able to predict microbial development patterns, automatically flag outlier test results, and assist with decision support for antimicrobial susceptibility testing [29]. Ultimately, using AI-powered analytics in conjunction with automated platforms decreases the number of manual

interventions, decreases variability between tests, and helps to ensure reproducibility of results from one laboratory to another [30]. Many challenges remain with regards to necessary substructure for AI, standardizing data from multiple laboratories, and incorporating AI into current laboratories. Furthermore, successful implementation of AI into practicing laboratories requires regulatory approval and validation. In summary, the merging of AI with molecular diagnostics as well as laboratory automation represents an important move forward to create intelligent and high-throughput systems for precision biological pathology [31].

7. Conclusion and Future Perspectives

AI tools enabling different types of artificial intelligence to enhance the accuracy of diagnosing microbiological diseases through pathology have had a significant impact on both laboratory diagnostic time and accuracy. Many AI-based machine learning and deep learning technologies are available for use in various types of digital pathology, including image analysis, microbial identification, molecular diagnostics, etc [32]. Generally, these tools can serve as supplemental to traditional pathologies in diagnosing microbiological infections by providing the clinician with complete or more detailed information regarding an individual patient through automated analysis of created images or image fragments. The benefits of utilizing AI tools are the reduced subjectivity when interpreting complex pathology data and microbiological data as well as timely provision of information to clinicians, particularly in the case of patients from resource-limited areas where they may be treated with inadequate or incorrect therapy due to limited availability of healthcare services [33]. The use of AI as a supplement to current diagnostic technologies (e.g., PCR technology, next-generation sequencing, MALDI-TOF MS, and laboratory automation technologies) has increased the success of pathogen monitoring, pathogen identification, and prediction of the emergence of drug-resistant pathogens during global malaria outbreaks. These advancements allow for the first stages of precision microbiology to occur via more rapid diagnosis, more tailored therapy, and better antimicrobial stewardship accessibility [34]. These tools, which are not to be understood as replacing the tarnished roles of microbiologists and pathologists, purport to be integrated as adjunct decision support systems, to augment and better diagnose confidence and consistency." While promising, to allow these tools to be used more clinically, an array of issues needs to be fixed first. These include, but are not limited to, the availability of new, annotated, quality datasets; the need for model transparency and interpretability; and the establishment of compliance frameworks in relation to model validation that are regulatory and ethical in their standards." Data security, system interoperability, and workforce training still remain as pivotal issues needing resolution as well. Large-scale clinical validation efforts, distinct specialized AI efforts, and multidisciplinary cooperation are the main outstanding areas to address in hopes of responsible AI refinement efforts. The impact of AI in the field of microbiological pathology and the diagnosis of infectious disease will be significant, rapid, and transformational [35].

8. Reference

1. World Health Organization. *Global Tuberculosis Report 2020*; World Health Organization: Geneva, Switzerland, 2020; Available online: https://www.who.int/tb/publications/global_report/en/ (accessed on 4 July 2024).
2. Schubert, A.M.; Kostrzynska, M. Advances in the molecular-based techniques for the detection of bacterial pathogens. *J. Microbiol. Methods* 2001, 47, 235–252.
3. Lamb, L.E.; Bartolone, S.N.; Ward, E.; Chancellor, M.B. Rapid detection of novel coronavirus (COVID-19) by reverse transcription-loop-mediated isothermal amplification. *PLoS ONE* 2020, 15, e0234682.
4. Mullis, K.; Faloona, F.; Scharf, S.; Saiki, R.; Horn, G.; Erlich, H. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harb. Symp. Quant. Biol.* 1986, 51, 263–273.

5. Corman, V.M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D.K.; Bleicker, T.; Brünink, S.; Schneider, J.; Schmidt, M.L.; et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020, 25, 2000045.
6. Dramé, M.; Tabue Teguo, M.; Proye, E.; Hequet, F.; Hentzien, M.; Kanagaratnam, L.; Godaert, L. Should RT-PCR be considered a gold standard in the diagnosis of COVID-19? *J. Med. Virol.* 2020, 92, 2312–2313.
7. Metzker, M.L. Sequencing technologies—The next generation. *Nat. Rev. Genet.* 2010, 11, 31–46.
8. Canto' n R, Ako' va M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM et al.: Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012, 18:413-431.
9. GBD 2017 Causes of Death Collaborators: Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018, 392:1736-1788.
10. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM et al.: The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016, 315:801-810.
11. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S et al.: Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020, 395:200-211.
12. Wang, P.X.; Sun, Y.; Li, X.; Wang, L.; Xu, Y.; He, L.L.; Li, G.L. Recent advances in dual recognition based surface enhanced Raman scattering for pathogenic bacteria detection: A review. *Anal. Chim. Acta* **2021**, 1157, 338279.
13. Andryukov, B.G.; Lyapun, I.N.; Matosova, E.V.; Somova, L.M. Biosensor technologies in medicine: From detection of biochemical markers to research into molecular targets (Review). *Mod. Technol. Med.* **2020**, 12, 71–85.
14. Zheng, L.; Qi, P.; Zhang, D. A simple, rapid and cost-effective colorimetric assay based on the 4-mercaptophenylboronic acid functionalized silver nanoparticles for bacteria monitoring. *Sens. Actuators B Chem* **2018**, 260, 983–989.
15. Percy N, Hu Y, Baker M, Maciel-Guerra A, Xue N, Wang W, Kaler J, Peng Z, Li F, Dottorini T. 2021. Genome-scale metabolic models and machine learning reveal genetic determinants of antibiotic resistance in *Escherichia coli* and unravel the underlying metabolic adaptation mechanisms. *mSystems* 6:e0091320. doi: 10.1128/mSystems.00913-20.
16. Nguyen M, Long SW, McDermott PF, Olsen RJ, Olson R, Stevens RL, Tyson GH, Zhao S, Davis JJ. 2019. Using machine learning to predict antimicrobial MICs and associated genomic features for nontyphoidal *Salmonella*. *J Clin Microbiol* 57:e01260-18. doi: 10.1128/JCM.01260-18.
17. Humphries RM, Bragin E, Parkhill J, Morales G, Schmitz JE, Rhodes PA. 2023. Machine-learning model for prediction of cefepime susceptibility in *Escherichia coli* from whole-genome sequencing data. *J Clin Microbiol* 61. doi: 10.1128/jcm.01431-22.
18. Smith KP, Wang H, Durant TJ, Mathison BA, Sharp SE, Kirby JE, Long SW, Rhoads DD. 2020. Applications of artificial intelligence in clinical microbiology diagnostic testing. *Clinical Microbiology Newsletter* 42:61–70. doi: 10.1016/j.clinmicnews.2020.03.006.
19. Cheng, J.Y.; Abel, J.T.; Balis, U.G.; McClintock, D.S.; Pantanowitz, L. Challenges in the development, deployment, and regulation of artificial intelligence in anatomic pathology. *Am. J. Pathol.* 2021, 191, 1684–1692.
20. Bagabir, S.A.; Ibrahim, N.K.; Bagabir, H.A.; Ateeq, R.H. Covid-19 and Artificial Intelligence: Genome sequencing, drug development and vaccine discovery. *J. Infect. Public Health* **2022**, 15, 289–296.
21. Chiu, H.-Y.R.; Hwang, C.-K.; Chen, S.-Y.; Shih, F.-Y.; Han, H.-C.; King, C.-C.; Gilbert, J.R.; Fang, C.-C.; Oyang, Y.-J. Machine learning for emerging infectious disease field responses. *Sci. Rep.* **2022**, 12, 328.

22. Pham, T.-H.; Qiu, Y.; Zeng, J.; Xie, L.; Zhang, P. A deep learning framework for high-throughput mechanism-driven phenotype compound screening and its application to COVID-19 drug repurposing. *Nat. Mach. Intell.* **2021**, *3*, 247–257.
23. Ong, E.; Wong, M.U.; Huffman, A.; He, Y. COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning. *Front. Immunol.* **2020**, *11*, 1581.
24. Ong, E.; Cooke, M.F.; Huffman, A.; Xiang, Z.; Wong, M.U.; Wang, H.; Seetharaman, M.; Valdez, N.; He, Y. Vaxign2: The second generation of the first Web-based vaccine design program using reverse vaccinology and machine learning. *Nucleic Acids Res.* **2021**, *49*, W671–W678.
25. Dutta, D.; Naiyer, S.; Mansuri, S.; Soni, N.; Singh, V.; Bhat, K.H. COVID-19 diagnosis: A comprehensive review of the RT-qPCR method for detection of SARS-CoV-2. *Diagnostics* **2022**, *12*, 1503.
26. Fournier, P.-E.; Drancourt, M.; Colson, P.; Rolain, J.-M. Modern clinical microbiology: New challenges and solutions. *Nat. Rev. Microbiol.* **2013**, *11*, 574–585.
27. Uddin, T.M.; Chakraborty, A.J.; Khushro, A.; Zidan, B.R.M.; Mitra, S.; Bin Emran, T.; Dhama, K.; Ripon, K.H.; Gajdacs, M.; Sahibzada, M.U.K.; et al. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *J. Infect. Public Health* **2021**, *14*, 1750–1766.
28. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.-D.; Coopersmith, C.M.; et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* **2016**, *315*, 801–810.
29. Smith, K.P.; Wang, H.; Durant, T.J.; Mathison, B.A.; Sharp, S.E.; Kirby, J.E.; Long, S.W.; Rhoads, D.D. Applications of artificial intelligence in clinical microbiology diagnostic testing. *Clin. Microbiol. Newsl.* **2020**, *42*, 61–70.
30. Nayak, D.S.K.; Mahapatra, S.; Routray, S.P.; Sahoo, S.; Sahoo, S.K.; Fouda, M.M.; Singh, N.; Isenovic, E.R.; Saba, L.; Suri, J.S.; et al. aiGeneR 1.0: An Artificial Intelligence Technique for the Revelation of Informative and Antibiotic Resistant Genes in *Escherichia coli*. *Front. Biosci.-Landmark* **2024**, *29*, 82.
31. Samantray, D.; Tanwar, A.S.; Murali, T.S.; Brand, A.; Satyamoorthy, K.; Paul, B. A Comprehensive Bioinformatics Resource Guide for Genome-Based Antimicrobial Resistance Studies. *OMICS A J. Integr. Biol.* **2023**, *27*, 445–460.
32. 148.Kumar Y., Koul A., Singla R., Ijaz M.F. Artificial intelligence in disease diagnosis: A systematic literature review, synthesizing framework and future research agenda. *J. Ambient. Intell. Humaniz. Comput.* 2023;14:8459–8486. doi: 10.1007/s12652-021-03612-z.
33. 149.Mirbabaie M., Stieglitz S., Frick N.R.J. Artificial intelligence in disease diagnostics: A critical review and classification on the current state of research guiding future direction. *Health Technol.* 2021;11:693–731. doi: 10.1007/s12553-021-00555-5.
34. 150.Arora G., Joshi J., Mandal R.S., Shrivastava N., Virmani R., Sethi T. Artificial intelligence in surveillance, diagnosis, drug discovery and vaccine development against COVID-19. *Pathogens.* 2021;10:1048. doi: 10.3390/pathogens10081048.
35. 151.Gerke S., Minssen T., Cohen G. Artificial Intelligence in Healthcare. Academic Press; Cambridge, MA, USA: 2020. Ethical and legal challenges of artificial intelligence-driven healthcare; pp. 295–336.