Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

THE ROLE OF NEXT-GENERATION SEQUENCING IN CLINICAL DIAGNOSTICS AND FUTURE PERSPECTIVES: A REVIEW OF THE LITERATURE

Mehboob Ali[*1], **Arslan Feroz**[2], **Hifsa Mobeen**[3], **Joun Abbas**[4]

[*1,2,4] *Department of Medical Laboratory Technology Allied Health Sciences, Superior University, Lahore.*
[3] *Faculty of Allied Health Sciences, Superior University, Lahore.*

[*1]bsmls-s22-032@superior.edu.pk,[2]bsmls-s22034@superior.edu.pk,[3]hifsamobeensuperior.edu.pk@gmail.com,
[4]abbasjoun32@gmail.com

DOI: https://doi.org/10.5281/zenodo.17862662

Keywords
NGS, Transformative technology

Article History
Received: 19 October 2025
Accepted: 24 November 2025
Published: 09 December 2025

Corresponding Author: *
Mehboob Ali

Abstract
The purpose of this study is to investigate the interplay of stress, emotional intelligence, and work-life balance among ambulance personnel. This study is a descriptive-correlational involving 120 ambulance paramedics personnel with at least 2 years of experience and were recruited from two regional ambulance services. Online self-assessment questionnaires were administered from April through June 2023. Descriptive statistics (i.e. frequency distribution, mean, SD) and inferential statistics to explore relationships between variables. Ambulance personnel work long, demanding hours (often exceeding 10 hours daily) with frequent night calls and weekend duties, leading to fatigue and moderate stress (average score 87.07). Though they display high emotional intelligence, their work-life balance suffers (score 41.26), particularly due to work intruding on their personal life. Higher emotional intelligence is linked to slightly increased stress but also slightly better work-life balance. Despite having a high emotional intelligence, ambulance personnel struggle with considerable stress and fatigue due to their busy schedules and poor work-life balance. Although there are links between these parameters, more study is still required. It is still essential for their performance and general well-being to prioritize sleep, breaks, and healthier work habits.

INTRODUCTION
NGS is a revolutionary DNA and RNA sequencing technology that allows the simultaneous sequencing of millions to billions of DNA fragments in a massively parallel manner. Introduced commercially around 2005 and originally called "massively parallel sequencing," NGS rapidly surpassed traditional Sanger sequencing by offering vastly higher throughput, speed, and cost-effectiveness. Unlike Sanger sequencing, which sequences DNA strands one at a time, NGS can analyze hundreds to thousands of genes or entire genomes in a single sequencing run, detecting a wide range of genomic features such as single nucleotide variants, copy number variants, structural mutations, and RNA fusions.[1, 2] The NGS process typically involves DNA or RNA extraction, fragmentation into smaller segments, attachment of special adapters, sequencing on a high-throughput platform, and sophisticated bioinformatics analysis to reconstruct the original sequences. This technology demands lower sample input, achieves higher accuracy, and can detect variants at much lower allele frequencies than

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

previous methods.[3] NGS has transformed genetic research and clinical diagnostics by enabling comprehensive and rapid analysis of genetic variation associated with diseases such as cancer, infectious diseases, and genetic disorders. It is also instrumental in fields like reproductive health, environmental studies, and evolutionary biology.[4]

Applications of NGS in Clinical Diagnostics

Infectious Disease Identification

NGS has become a vital tool for infectious disease identification due to its ability to sequence large amounts of genetic material rapidly and accurately, without relying on traditional culture methods. NGS can identify a broad range of pathogens—including viruses, bacteria, fungi, and parasites—directly from clinical samples through approaches like whole-genome sequencing, targeted sequencing, and metagenomics. Whole-genome sequencing reconstructs complete microbial genomes, aiding in pathogen discovery, outbreak tracking, and antimicrobial resistance profiling. Targeted NGS focuses on specific genetic regions, such as 16S rRNA for bacteria, enabling sensitive and specific detection of pathogen species.[5, 6]

Metagenomic sequencing analyzes total DNA or RNA from a sample, enabling detection of known and novel organisms without prior knowledge. These methods provide enhanced sensitivity, allowing detection of low abundance pathogens and mixed infections. NGS also supports epidemiological investigations and antibiotic stewardship by revealing resistance genes. Despite some challenges like data complexity and cost, NGS is increasingly integrated into clinical microbiology for rapid, precise infectious disease diagnostics, transforming patient management and public health responses. Its expanding role promises improved diagnostic accuracy and comprehensive pathogen surveillance in the future.[7, 8]

Genetic Disorder Diagnosis

NGS has revolutionized the diagnosis of genetic disorders by enabling comprehensive and high-throughput analysis of the genome. It allows sequencing of the entire genome (whole-genome sequencing), coding regions (whole-exome sequencing), or targeted gene panels associated with specific phenotypes. This capacity facilitates the detection of a wide range of genetic variations, including single nucleotide variants (SNVs), insertions/deletions (INDELs), copy number variations (CNVs), and structural rearrangements, which can underlie inherited and rare genetic diseases. Unlike traditional methods such as Sanger sequencing, NGS can analyze multiple genes simultaneously, increasing diagnostic yield and reducing time and cost.[9]

NGS assists in early and accurate diagnosis even for complex or genetically heterogeneous disorders by identifying pathogenic mutations that might be missed otherwise. It also supports discovery of novel mutations and better phenotype-genotype correlations, which improves classification and informs prognosis.[10] Clinicians increasingly rely on NGS for patient care decisions, including prenatal diagnosis, genetic counseling, and personalized treatment plans. Despite some challenges like data interpretation and incidental findings, ongoing improvements in sequencing technology, bioinformatics pipelines, and clinical guidelines are enhancing its diagnostic utility and integration into routine genetic testing for diverse inherited conditions.[11]

Oncology and Cancer Genomics

NGS has significantly transformed oncology and cancer genomics by enabling comprehensive genomic profiling of tumors at unprecedented depth and scale. This technology allows the identification of somatic and germline mutations, structural variations, and other genetic alterations that drive cancer development and progression. NGS facilitates personalized cancer care by revealing actionable mutations, guiding targeted therapies, and monitoring minimal residual disease and treatment resistance. It also supports the identification of hereditary cancer syndromes, enabling preventive strategies for at-risk individuals. The ability of NGS to analyze multiple genes simultaneously reduces diagnostic time and enhances sensitivity compared to traditional testing methods.[12, 13]

Emerging advancements such as liquid biopsies and single cell sequencing further improve the precision of cancer detection and monitoring by analyzing circulating tumor DNA and cellular heterogeneity within tumors. Despite challenges like data

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

complexity and cost, NGS is increasingly integrated into routine clinical practice for various cancer types including lung, breast, colorectal, and melanoma, underscoring its critical role in precision oncology and improved patient outcomes.[14, 15]

Table 1. Advantages of NGS

Advantage	Description
High-throughput	Allows simultaneous sequencing of millions of DNA fragments.
High sensitivity	Detects rare mutations and low-frequency variants.
Multi-gene analysis	Can analyze multiple genes or whole exomes/genomes at once.
Faster results	Reduces turnaround time for diagnostics.
Comprehensive coverage	Detects SNVs, CNVs, structural variations, and fusions.

Emerging Trends and Innovations in NGS

Emerging trends in NGS increasingly focus on rapid and portable sequencing technologies alongside multi-omics integration, which are poised to revolutionize clinical and research applications. Rapid sequencing platforms aim to reduce turnaround times drastically, enabling real-time genomic data generation crucial for timely diagnostics, especially in infectious diseases and oncology. Portable sequencers, such as those developed by Oxford Nanopore Technologies, bring sequencing capabilities directly to point-of-care and field settings, facilitating faster decision-making in diverse environments, including remote or resource-limited areas.[2, 16]

These innovations are complemented by advances in multi-omics integration, which combines genomic, transcriptomic, epigenomic, proteomic, and metabolomic data to provide a holistic view of biological systems. This multi-layered approach deepens understanding of disease pathogenesis and patient heterogeneity, enabling more precise diagnostics and personalized therapeutics.[17] The fusion of these data types with advanced bioinformatics, artificial intelligence, and machine learning tools enhances the interpretation of complex datasets, turning raw sequence information into actionable clinical insights. Continued advancements in automation, miniaturization, and data analytics are expected to make NGS more accessible, efficient, and impactful for broader applications in precision medicine, real-time disease monitoring, and population health.[18, 19]

Integration of NGS with Bioinformatics and Artificial Intelligence

NGS generates vast and complex datasets requiring sophisticated bioinformatics tools for accurate analysis and interpretation. The integration of NGS with bioinformatics and artificial intelligence (AI) has become essential to unlock the full potential of sequencing data. Advanced bioinformatics workflows enable quality control, sequence alignment, variant calling, annotation, and visualization, transforming raw data into clinically actionable insights.[19] Recent developments include AI and machine learning algorithms that enhance variant interpretation, pattern recognition, and predictive modeling, helping to distinguish pathogenic mutations from benign variants efficiently. With growing adoption of long-read sequencing technologies, bioinformatics tools increasingly support complex structural variant detection and pan-genome analyses.[2]

The combined power of cloud computing and federated AI facilitates scalable data processing and collaborative genomics research across institutions while maintaining data privacy. This integration also accelerates discoveries in cancer genomics, rare diseases, infectious diseases, and pharmacogenomics by enabling rapid, high-throughput, accurate interpretation tailored to clinical needs. Challenges remain in managing data volume, standardizing pipelines, and training skilled bioinformaticians; however, ongoing innovation is making NGS analyses more accessible to clinicians and researchers. As NGS technologies evolve, synergistic advancements in bioinformatics and AI are critical to realizing personalized medicine and broad genomic applications in healthcare and beyond.[20]

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

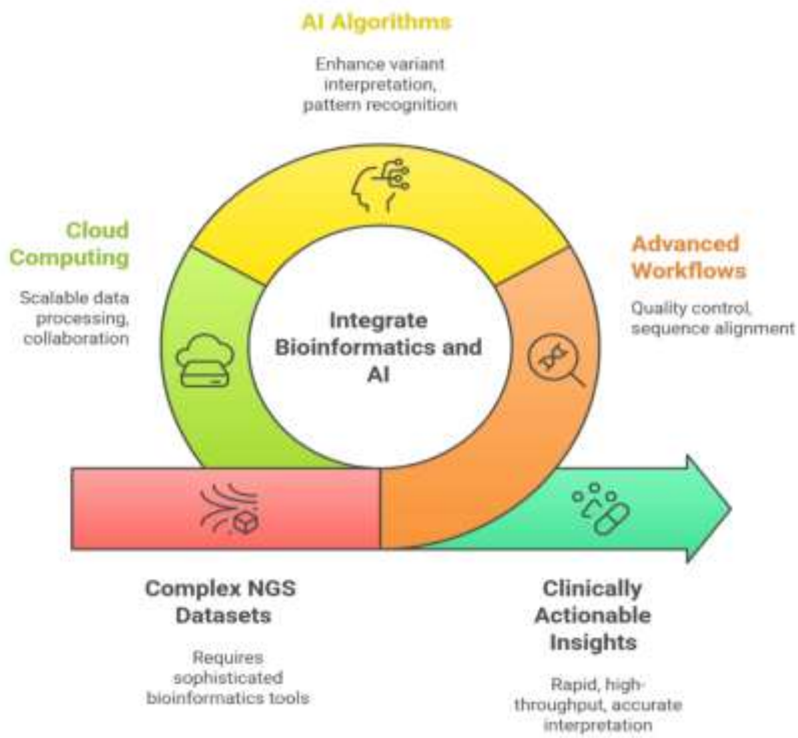

Figure 1: Unlocking NGS potential with Bioinformatics and AI

MATERIAL AND METHODS

4.1 Study Design: This research was conducted as a **Narrative Review**, focusing on published studies related to the applications of Next-Generation Sequencing (NGS) in clinical diagnostics and its future perspectives.

4.2 Settings: The review was carried out in the**Superior University Lahore**, using digital databases and online scientific resources.

4.3 Study Duration: The review work was completed over **4 months** following the approval of the synopsis.

4.4 Sample Size: As this is a review, no patients were included. Instead, **relevant published articles** were selected based on inclusion criteria. A total of **40–60 peer-reviewed articles** from 2020–2025 were reviewed.

4.5 Sample Selection

4.5.1 Inclusion Criteria

- Articles published in English.
- Studies between **2010–2025**.
- Peer-reviewed research papers, reviews, systematic reviews, meta-analyses, and technical reports.
- Studies focusing on NGS in clinical diagnostics (oncology, infectious diseases, genetic disorders, etc.).
- Studies discussing future applications or technological advancements in NGS.

4.5.2 Exclusion Criteria

- Articles not related to NGS or not relevant to clinical applications.
- Studies published before 2010.
- Non-English publications.
- Editorials, conference abstracts without full data, and non-peer-reviewed materials.
- Duplicated or low-quality studies.

Data analysis procedure

Data collected from the selected literature were analyzed using a qualitative and descriptive approach, as this study was conducted as a narrative review. All extracted information was organized in Microsoft Excel 2021, and Mendeley Reference Manager was

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

used to manage citations and remove duplicates. The analysis involved summarizing key findings and categorizing studies into themes such as NGS applications, diagnostic accuracy, mutation detection, pathogen identification, technological advancements, limitations, and future perspectives. No statistical tests or p-values were applied because no primary data were collected. Instead, the data were narratively synthesized to highlight patterns, trends, and gaps in the existing evidence.

DISCUSSION

Next-Generation Sequencing (NGS) has rapidly transformed the landscape of clinical diagnostics by enabling high-throughput, precise, and cost-effective analysis of genetic material. The present review highlights the significant impact of NGS on detecting genetic mutations, identifying pathogens, and enhancing patient care, which is consistent with several previous studies. NGS allows simultaneous sequencing of multiple genes, significantly improving diagnostic yield in oncology and inherited disorders compared to traditional Sanger sequencing. Similarly, NGS provides a comprehensive molecular profile of tumors, aiding in the identification of actionable mutations and facilitating personalized therapy. These findings align closely with the present review, which also underscores the superior sensitivity and specificity of NGS in clinical diagnostics.

In infectious disease diagnostics, the role of NGS has been particularly transformative. Traditional culture-based methods often fail to detect fastidious or novel pathogens, whereas metagenomic NGS can identify a wide range of microorganisms directly from patient samples. NGD-based metagenomic sequencing successfully identified rare pathogens in cerebrospinal fluid samples, leading to timely clinical intervention. The current review supports this observation, highlighting studies where NGS enabled rapid detection of viral, bacterial, and fungal infections, including cases where conventional methods were inconclusive.

Role of NGS in Real-Time Disease Surveillance and Pandemic Response

NGS plays a pivotal role in real-time disease surveillance and pandemic response by enabling rapid, high-resolution genomic analysis of pathogens directly from clinical and environmental samples. Its high sensitivity and capacity to generate comprehensive whole-genome data allow early detection of emerging infectious agents, tracking of transmission chains, and identification of mutations linked to increased virulence or antimicrobial resistance. NGS has revolutionized public health efforts, exemplified by its use in initiatives like the CDC's PulseNet system for foodborne outbreak detection, the WHO's influenza surveillance networks, and in-field rapid sequencing during Ebola outbreak in West Africa.[21, 22]

Portable sequencing technologies facilitate onsite genome monitoring, providing near real-time data that guide outbreak control strategies without the need to transport samples to distant laboratories. Furthermore, the standardized sharing and analysis of NGS data globally enhance coordinated responses to pandemics, enabling timely vaccine strain selection and targeted public health interventions. Despite challenges such as infrastructure, cost, and workforce training, integrating NGS into routine surveillance enhances the precision and speed of epidemic management. The future of disease surveillance relies on combining NGS with digital health platforms and AI analytics to provide actionable insights, improve outbreak predictions, and mitigate the impact of infectious diseases worldwide.[23, 24]

Current Challenges and Limitations in Clinical NGS

NGS faces several significant challenges and limitations in clinical settings despite its transformative potential. One major hurdle is the high cost associated with NGS platforms, reagents, maintenance, and the required computational infrastructure, which limits accessibility, especially in smaller labs or resource-constrained environments.[25] The massive volume of data generated necessitates robust storage solutions and considerable computational power, presenting logistical and financial burdens. Skilled bioinformaticians are essential for accurate data analysis, yet there is a shortage of expertise, creating bottlenecks in interpreting complex sequencing results.[26, 27]

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

Clinical interpretation of genetic variants remains particularly difficult due to the presence of variants of unknown significance (VUS), which can cause uncertainty in patient management. Ethical, legal, and social concerns also arise around data privacy, informed consent, and incidental findings that carry implications beyond the initial diagnostic question. Additionally, tissue availability and tumor heterogeneity affect sample quality and the representativeness of sequencing results, complicating cancer diagnostics. Turnaround times are often extended by the multi-step testing process, and insurance reimbursement for NGS tests can be inconsistent or limited. Variability in panel design, reporting standards, and regulatory requirements add further complexity to clinical implementation. Overcoming these challenges requires multidisciplinary collaboration, continuous technological advances, standard guidelines, and expanded education for healthcare providers to fully harness NGS's potential in personalized medicine.[2, 28, 29]

CONCLUSION

Next-Generation Sequencing (NGS) has emerged as a transformative tool in clinical diagnostics, offering unparalleled precision, speed, and breadth in detecting genetic mutations, pathogens, and inherited disorders. Its applications have significantly enhanced diagnostic accuracy, facilitated personalized treatment strategies, and improved patient management across multiple medical disciplines. Despite challenges such as high costs, data interpretation complexities, and ethical considerations, NGS continues to demonstrate immense potential for shaping the future of medicine. The study emphasizes that ongoing technological advancements and integration with bioinformatics will further expand its clinical utility, making NGS a cornerstone of modern diagnostic and precision medicine practices.

RECOMMENDATION

Based on this review, healthcare institutions should adopt Next-Generation Sequencing (NGS) more widely to improve diagnostic accuracy and support personalized medicine. Training programs for laboratory staff and clinicians are needed to strengthen molecular and bioinformatics skills, while efforts to reduce NGS costs can help increase patient access. Standardized protocols and regulatory guidelines are essential for ensuring quality and reliability, and ethical issues such as data privacy must be clearly addressed. Continued research, including the use of AI and advanced sequencing technologies, should be encouraged, along with awareness and education to promote proper understanding and use of NGS.

LIMITATIONS

This review has limitations because it is based only on published English-language studies, which may miss important evidence and introduce bias. It also lacks a meta-analysis and includes studies with varying designs and methods, making direct comparison and strong conclusions more difficult.

REFERANCES

1. Katara A, Chand S, Chaudhary H, Chaudhry V, Chandra H, Dubey RC. Evolution and applications of Next Generation Sequencing and its intricate relations with chromatographic and spectrometric techniques in modern day sciences. Journal of Chromatography Open. 2024;5:100121.
2. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, et al. Next-generation sequencing technology: current trends and advancements. Biology. 2023;12(7):997.
3. Tsai CN. The Invention of Next-Generation Sequencing. Berkeley Tech LJ. 2024;39:613.
4. Kulski J. Next-generation sequencing—an overview of the history, tools, and "Omic" applications. Next generation sequencing-advances, applications and challenges. 2016:3-60.
5. Roy S, Hartley J, Dunn H, Williams R, Williams CA, Breuer J. Whole-genome sequencing provides data for stratifying infection prevention and control management of nosocomial influenza A. Clinical Infectious Diseases. 2019;69(10):1649-56.
6. Gwinn M, MacCannell D, Armstrong GL. Next-generation sequencing of infectious pathogens. Jama. 2019;321(9):893-4.

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

7. Elbehiry A, Abalkhail A. Metagenomic next-generation sequencing in infectious diseases: clinical applications, translational challenges, and future directions. Diagnostics. 2025;15(16):1991.
8. Zhao Y, Zhang W, Zhang X. Application of metagenomic next-generation sequencing in the diagnosis of infectious diseases. Frontiers in Cellular and Infection Microbiology. 2024;14:1458316.
9. Schuler BA, Nelson ET, Koziura M, Cogan JD, Hamid R, Phillips JA. Lessons learned: next-generation sequencing applied to undiagnosed genetic diseases. The Journal of clinical investigation. 2022;132(7).
10. Lohmann K, Klein C. Next generation sequencing and the future of genetic diagnosis. Neurotherapeutics. 2014;11(4):699-707.
11. Di Resta C, Galbiati S, Carrera P, Ferrari M. Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities. Ejifcc. 2018;29(1):4.
12. Ghoreyshi N, Heidari R, Farhadi A, Chamanara M, Farahani N, Vahidi M, et al. Next-generation sequencing in cancer diagnosis and treatment: clinical applications and future directions. Discover Oncology. 2025;16(1):578.
13. Hussen BM, Abdullah ST, Salihi A, Sabir DK, Sidiq KR, Rasul MF, et al. The emerging roles of NGS in clinical oncology and personalized medicine. Pathology-Research and Practice. 2022;230:153760.
14. Guan Y-F, Li G-R, Wang R-J, Yi Y-T, Yang L, Jiang D, et al. Application of next-generation sequencing in clinical oncology to advance personalized treatment of cancer. Chinese journal of cancer. 2012;31(10):463.
15. Kim JW, Na HY, Lee S, Kim J-W, Suh KJ, Kim SH, et al. Clinical implementation of next-generation sequencing testing and genomically-matched therapy: a real-world data in a tertiary hospital. Scientific Reports. 2025;15(1):2171.
16. Davies K. The $1,000 genome: the revolution in DNA sequencing and the new era of personalized medicine: Simon and Schuster; 2015.
17. Kumar D. Integrated Genomics and Multi-OMICS in Precision and Personalised Medicine: Emerging New Paradigm for Clinical Medicine. Apollo Medicine. 2025:09760016251324360.
18. Ikwelle TA, Ihim AC, Ozuruoke DF, Obi PC, Obi CU, Onuora IJ, et al. Multi-Omics Integration in Personalized Medicine: Advancing Laboratory Diagnostics and Precision Therapeutics in the Era of Individualized Healthcare. Journal of Drug Delivery & Therapeutics. 2025;15(5).
19. Nam Y, Kim J, Jung S-H, Woerner J, Suh EH, Lee D-g, et al. Harnessing artificial intelligence in multimodal omics data integration: paving the path for the next frontier in precision medicine. Annual review of biomedical data science. 2024;7.
20. Alvarellos M, Sheppard HE, Knarston I, Davison C, Raine N, Seeger T, et al. Democratizing clinical-genomic data: How federated platforms can promote benefits sharing in genomics. Frontiers in Genetics. 2023;13:1045450.
21. Farkas K, Williams RC, Hillary LS, Garcia-Delgado A, Jameson E, Kevill JL, et al. Harnessing the power of next-generation sequencing in wastewater-based epidemiology and global disease surveillance. Food and Environmental Virology. 2025;17(1):5.
22. Basirpour B, Tabaripour R, Jafarian F, Fakhar M, Hezarjaribi HZ, Gholami S. Applications of the powerful next-generation sequencing tool for detecting parasitic infections: towards a smart laboratory platform. Microbial Pathogenesis. 2025:107746.
23. Long J, Zeng B, Li J, Zhang J, Deng G. Global trends in the application of nanopore sequencing technology in the detection of infectious disease pathogens: A bibliometric analysis from 2014 to 2024. Frontiers in Medicine. 2025;12:1610063.
24. Rizzo JM, Buck MJ. Key principles and clinical applications of “next-generation” DNA sequencing. Cancer prevention research. 2012;5(7):887-900.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

25. Abbasi A, Alexandrov LB. Significance and limitations of the use of next-generation sequencing technologies for detecting mutational signatures. DNA repair. 2021;107:103200.
26. Messner DA, Koay P, Naber JA, Cook-Deegan R, Majumder M, Javitt G, et al. Barriers to clinical adoption of next-generation sequencing: a policy Delphi panel's solutions. Personalized medicine. 2017;14(4):339-54.
27. Daber R, Sukhadia S, Morrissette JJ. Understanding the limitations of next generation sequencing informatics, an approach to clinical pipeline validation using artificial data sets. Cancer genetics. 2013;206(12):441-8.
28. Cheng C, Fei Z, Xiao P. Methods to improve the accuracy of next-generation sequencing. Frontiers in bioengineering and biotechnology. 2023;11:982111.
29. Yadav D, Patil-Takbhate B, Khandagale A, Bhawalkar J, Tripathy S, Khopkar-Kale P. Next-Generation sequencing transforming clinical practice and precision medicine. Clinica Chimica Acta. 2023;551:117568.
30. Popova L, Carabetta VJ. The use of next-generation sequencing in personalized medicine. High Throughput Gene Screening: Methods and Protocols. 2024:287-315.