DOI: 10.7860/JCDR/2025/78052.20832

Review Article

Genetics Section

Applications and Challenges of Advanced Molecular Diagnostics in Clinical Microbiology and Epidemiology: A Literature Review

UJWALA DAMINENI[1], AMULYA VARSHINI BANKA[2], SRI RAM CHARAN GUNDAPANENI[3], NEHA NARAYAN[4], YETHINDRA VITYALA[5]

ABSTRACT

Molecular biology techniques have revolutionised biomedical research and clinical practice, enabling the detailed examination of genetic information and cellular processes. In epidemiology and surveillance, molecular approaches have advanced more rapidly than in clinical use and are recognised for their superior discrimination. This review aimed to provide details on the clinical and epidemiological applications of molecular diagnostics, particularly Whole Genome Sequencing (WGS), in various bacterial and viral diseases and to discuss its future prospects. A comprehensive literature review was conducted using PubMed, Scopus and the Web of Science from January 2002 to December 2024. This review found that WGS offers advantages for antibiotic resistance surveillance and can be used as a standard to evaluate antibiotic susceptibility in pathogenic bacteria. In Tuberculosis (TB), WGS has transformed molecular epidemiology and effectively identifies transmission clusters. Next Generation Sequencing (NGS) exceeds traditional methods for detecting viral pathogens, including novel ones, and outperforms Sanger sequencing for detecting low-frequency antiviral resistance mutations. Metagenomics identifies all potential pathogens in a single test using NGS of DNA, surpassing traditional diagnostics. NGS provides a methodological foundation for investigating bacterial transmission in forensic microbiology. The implementation of WGS in clinical and epidemiological settings remains inconsistent, with varying applications across countries and contexts. Although WGS offers advancements in fastidious microbes, plasmid-mediated resistance detection, and comprehensive characterisation, its routine use depends on overcoming challenges, targeting diseases and demonstrating benefits. Challenges such as a lack of standardisation in bioinformatics analysis, incomplete mutation catalogs and technical complexities hinder its routine use. However, advancements in mutation catalogs and the optimised use of WGS may enable comprehensive and accurate diagnosis, leading to personalised treatment strategies. A significant shift is expected in developed countries within five years, driven by global sample preparation and result analysis approaches. Developing countries face challenges that complicate their efforts, while developed nations have made progress. Future improvements in mutation catalogs and the optimised use of WGS may enable comprehensive and accurate diagnosis, leading to personalised treatment strategies.

Keywords: Antibiotic resistance, Bioinformatics, Tuberculosis, Whole-genome sequencing

INTRODUCTION

Molecular biology techniques have revolutionised biomedical research and clinical practice by enabling the detailed examination of genetic information and cellular processes. These methods include DNA sequencing, gene expression analysis, gene cloning, genetic manipulation and recombinant protein production [1]. Clinically, molecular diagnostics employ in situ hybridisation, Southern blot analysis and Polymerase Chain Reaction (PCR) for disease diagnosis and monitoring [2]. Since the 1980s, molecular pathology has evolved from single-gene evaluations to comprehensive analysis of exomes and genomes in complex genetic disorders [3]. This shift has accelerated the identification of mutations that cause genetic diseases and cancers. Consequently, these molecular techniques offer new opportunities for diagnosis, staging, prognosis and treatment across medical specialties, including orthopaedics and microbiology [1,4]. These advancements have enabled personalised treatment strategies based on genetic profiles and are expected to significantly influence both surgical and non surgical decisions [1]. The use of real-time PCR in hospitals is gradual and region-dependent, leading to its implementation in the United Kingdom. Some reference centres in developed countries have yet to adopt it, whereas in developing nations, targeted applications for complex or urgent issues are under consideration [5].

In epidemiology and surveillance, molecular approaches have advanced more rapidly than in clinical use and are recognised for their superior discrimination [6]. The WGS also shows high discriminatory capabilities but faces adoption barriers [7,8]. Bioinformatics and phylogenetic analysis are complex and often require culturing due to the increased complexity and cost of direct sampling. However, sequencing from cultures is becoming more cost-effective. Applications vary among microorganisms. Advancements in TB have led to the replacement of cultures in some countries [9]. Implementation for other bacteria, particularly those causing less common diseases with unknown resistance genetics or involving consortia of pathogens, remains unestablished. This review aimed to provide details on clinical and epidemiological applications in various bacterial and viral diseases and to discuss future prospects.

Methodology

A comprehensive literature review using PubMed, Scopus and Web of Science was conducted on molecular diagnostics, particularly WGS, in clinical microbiology, epidemiology, antibiotic resistance and infectious disease management, from January 2002 to December 2024. Peer-reviewed studies, reviews and reports meeting the inclusion criteria were analysed, whereas unrelated research, non English publications, and studies lacking primary or secondary data were excluded. Information was extracted from the WGS applications to identify antibiotic resistance and TB, NGS for detecting viral pathogens, metagenomics in disease surveillance and outbreak investigations and global challenges

and opportunities for adopting WGS. Data were categorised into thematic areas, emphasising the impact of molecular technology on clinical diagnosis and epidemiology, focusing on established applications, benefits and implementation challenges.

Role in Clinical Diagnosis

Antibiotic-resistant microorganisms pose a significant threat to global health [10]. Currently, some microorganisms are resistant to all available antibiotics, causing an estimated 700,000 deaths annually, and this number is expected to rise to over 10 million by 2050, surpassing those of cancer and heart disease [11]. Antibiotic resistance is encoded by point mutations in chromosomes, plasmids and other mobile genetic elements, including all genes. Bacteria exhibit varied resistance patterns with distinct clinical implications, prompting organisations such as the European Centre for Disease Prevention and Control, World Health Organisation (WHO) and Centers for Disease Control and Prevention (CDC) to list diseases and resistance types that require strict monitoring [12,13].

The NGS offers several advantages for surveillance. It can be used as a standard to evaluate antibiotic susceptibility in pathogenic bacteria [14]. A significant challenge in clinical implementation is the need for rapid bioinformatics analysis. Tools such as SRST2 and ARIBA address this issue by providing detailed characterisation of isolates and resistance without extensive bioinformatics expertise, yielding information on species, Multi-Locus Sequence Types (MLST), and resistance genes soon after sequencing [15,16].

The integration of Genomic Sequencing (GS) into clinical microbiology laboratories is facilitated by its dual relevance in clinical practice and epidemiology. Training, equipment acquisition and familiarisation with the procedures are essential for surveillance purposes.

Bacterial Pathology

Tuberculosis (TB), the leading global infectious disease, accounts for approximately 10 million new cases and 1.8 million fatalities annually, as reported by the WHO [17,18]. Coronavirus Disease-2019 (COVID-19) pandemic has significantly hindered access to TB diagnosis and treatment by 2021 [19,20]. That year, global TB infections rose by 4.5 percent to 10.6 million, resulting in 1.6 million deaths, of which 187,000 were Human Immunodeficiency Virus (HIV)-positive individuals, effectively negating progress in reducing TB mortality worldwide [21]. *Mycobacterium tuberculosis* acquires resistance through point mutations. The WGS demonstrated comparable or superior sensitivity and specificity to culture methods for detecting rifampicin and isoniazid resistance, though results for other antibiotics varied. Since 2017, some public health facilities have replaced phenotypic testing with WGS for *Mycobacterium tuberculosis* complex diagnosis and resistance profiling [9].

WGS has transformed the molecular epidemiology of TB [22]. It effectively identifies transmission clusters and parallels conventional typing methods. Wyllie DH et al., and Stucki D et al., found that the Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat technique overestimates recent United Kingdom transmissions, particularly among immigrants [23,24]. WGS aligns better with epidemiological studies and can detect transmission across countries [25].

However, WGS faces challenges such as a lack of standardisation in bioinformatics analysis and incomplete mutation catalogs, with rare variants remaining problematic [26]. Databases such as the Comprehensive Analysis Server for the *Mycobacterium tuberculosis* complex, TB Profiler, and PhyReSE catalog known mutations and enable automated sequence analysis, whereas consortia such as the Comprehensive Resistance Prediction for TB: an International Consortium and ReSeqTB advance the discovery of new diagnostic mutations [27-29]. Future improvements in mutation catalogs and the optimised use of WGS may enable comprehensive and accurate diagnosis, leading to personalised treatment for patients with multidrug-resistant and extensively drug-resistant TB [30].

Viral Pathology

The NGS exceeds traditional methods for detecting viral pathogens, including novel ones, in samples lacking prior genomic information. Analysing the viral components of the microbiota is technically challenging, complicating virus identification in complex samples and distinguishing colonisation from infection [31]. Addressing viral contaminants in materials and reagents is therefore critical [32]. Despite these challenges, NGS has identified pathogens such as rubella virus in cases of ophthalmitis and mumps virus in cerebrospinal fluid from patients where conventional methods failed [33,34].

Studies have shown that NGS outperforms Sanger sequencing in detecting low-frequency antiviral resistance mutations [35]. Primarily used for HIV, it is also employed for Hepatitis C, cytomegalovirus, hepatitis B, hepatitis A and influenza viruses [36-41]. Current HIV resistance guidelines exclude the routine identification of minority mutations [42], limiting its use to research, although it may be considered for non nucleoside reverse transcriptase inhibitors. Limited data on treatment failure correlation restrict frequent testing; however, the transmission potential is significant [43,44].

RNA viruses that cause persistent infections, such as HIV and Hepatitis C virus, exhibit high mutation rates, revealing the viral population dynamics over time [45,46]. Compartmentalisation, mutations, and varying drug accessibility can lead to treatment failures [45,46]. The ability of NGS to analyse viral populations over time is advantageous, revealing that co-infection or superinfection is linked to high-risk behaviours [47]. This method also aids in the study of viral transmission among patients, although conclusions drawn from sequence analysis alone, without supplementary clinical or epidemiological data, can be challenging [48].

NGS data have meticulously analysed transmission networks and viral epidemics in epidemiological and forensic contexts, as demonstrated by real-time assessments of Ebola, Zika and chikungunya outbreaks using third-generation sequencing [49-51]. However, identifying viral transmission between specific individuals and their directionality based solely on sequencing data should be approached cautiously because of conflicting findings and methodological concerns.

Nosocomial Infections

WGS is an advanced epidemiological investigation method [52]. A hospital faced a potential outbreak, necessitating verification due to the high mortality among patients and probable links to underlying conditions. Of the estimated 20 potential cases, only 12 samples from 6 patients were analysed. Genomic Sequencing (GS) and phylogenetic analysis confirmed that all cases were of the same isolate type, with minor genome-wide variations. MLST identified it as ST175, a common global sequence type, complicating conclusions about a single origin based on these data alone [53]. Multiple samples per patient are crucial for assessing infections caused by different clones.

Whole-genome phylogenetic analysis of complex cases confirmed outbreaks, identified affected individuals, assessed the scope and determined infection sources. For instance, a major epidemic in a hospital affected 64 individuals. With increasing rates of *Pseudomonas aeruginosa* infection, samples from different wards were examined. This approach confirmed the spread of the outbreak and revealed a more complex evolutionary structure than a single outbreak, indicating the presence of minor epidemics.

Metagenomics in Clinical Diagnosis and Disease Monitoring

Metagenomics identifies all potential pathogens in a single test through NGS of DNA, thereby surpassing traditional diagnostics. Neuroleptospirosis was successfully diagnosed in a critically ill patient, leading to effective treatment and recovery [54].

Metagenomics can be used to identify specific strains, mutations, resistance genes and virulence factors. It has been utilised to study bacterial outbreaks, trace origins and transmission and characterise microbiomes in various human organs and tissues linked to acute and chronic conditions [55,56]. Dysbiosis is associated with diseases such as diabetes, Crohn's disease and Alzheimer's disease [57,58]. Metagenomics aids in the management and monitoring of these disorders, as demonstrated by its use in treating *Clostridium difficile* infections via fecal transplantation [59].

Currently, metagenomics is too complex and expensive for routine clinical practice. Most studies have been conducted in research settings with evolving methodologies, hindering their adoption in public health systems that require validation. As technical challenges are resolved and clinical applications expand, metagenomics is anticipated to replace many existing microbiological methods in the near future [60].

Role of Sequencing in Forensic Microbiology

Forensic microbiology gained prominence after the 2001 anthrax bioterrorist attack in the United States, in which NGS was used without modern GS [61,62]. NGS had not been applied to bacterial transmission in forensic microbiology until recently, owing to legal constraints or the novelty of the technology.

Researchers have conducted studies involving *Neisseria gonorrhoeae* transmission, where WGS is used for strains from the suspect, victim, and three local controls [63]. Traditional techniques such as MLST and pulsed-field gel electrophoresis lack sufficient discriminatory power [6]. MLST could not differentiate between the case and control strains, as all strains belonged to the same ST9363 strain. Although pulsed-field gel electrophoresis is typically more discriminatory than MLST, it also failed to distinguish between the case and control strains [6,63].

Francés-Cuesta C et al., used methods to align sequences with a genetically similar reference genome, confirming that strains from the suspect and victim were identical, while the control strain differed by two nucleotides [63]. The control patient had no known connection with the case individuals, suggesting a shared infection source for the control and suspect patients. Although GS does not determine the transmission route, detailed case information clarifies it. This pioneering use of NGS in forensic microbiology provides a methodological foundation for investigating bacterial transmission in a forensic context.

CONCLUSION(S)

The implementation of WGS in clinical and epidemiological settings remains inconsistent, with varying applications across countries and contexts, such as its systematic use in the United Kingdom, foodborne pathogen outbreaks, and differing adoption rates depending on the disease. Third-generation sequencers enable faster diagnosis by providing real-time genomic data from individual molecules, albeit at high error rates. WGS is valuable in surveillance, epidemiology and clinical microbiology; however, its routine use depends on overcoming challenges, targeting diseases and demonstrating benefits. While phenotypic or molecular methods can quickly identify some pathogens, WGS offers advancements in the detection of fastidious microbes, plasmid-mediated resistance, and comprehensive characterisation. A major shift is expected in developed countries within five years, driven by global sample preparation and result-analysis approaches. Despite their potential to address prevalent endemic diseases, developing countries face challenges that complicate efforts, while developed nations have made progress.

REFERENCES

[1] Evans CH, Rosier RN. Molecular biology in orthopaedics: The advent of molecular orthopaedics. Bone Joint Surg Am. 2005;87(11):2550-64. Doi: 10.2106/JBJS.E.00019. PMID: 16264134.

[2] Aziz KJ. Clinical molecular biology: Concepts and applications. Adv Clin Chem. 1996;32:39-72. Doi: 10.1016/s0065-2423(08)60425-4. PMID: 8899070.

[3] Bayrak-Toydemir P, Wooderchak-Donahue W. Gene/genome mutation detection and testing. In book: Pathobiology of Human Disease. 2014; pp. 3408-3417. Doi: 10.1016/b978-0-12-386456-7.06603-x.

[4] Weile J, Knabbe C. Current applications and future trends of molecular Diagnostics in clinical bacteriology. Anal Bioanal Chem. 2009;394(3):731-42. Doi: 10.1007/s00216-009-2779-8. PMID: 19377839; PMCID: PMC7079892.

[5] Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, et al. Real-time, portable genome sequencing for Ebola surveillance. Nature. 2016;530(7589):228-32. Doi: 10.1038/nature16996. PMID: 26840485; PMCID: PMC4817224.

[6] Maiden MC. Multilocus sequence typing of bacteria. Annu Rev Microbiol. 2006;60:561-88. Doi: 10.1146/annurev.micro.59.030804.121325. PMID: 16774461.

[7] Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol. 2019;14:319-38. Doi: 10.1146/annurev-pathmechdis-012418-012751. Epub 2018 Oct 24. PMID: 30355154; PMCID: PMC6345613.

[8] Rossen JWA, Friedrich AW, Moran-Gilad J; ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD). Practical issues in implementing whole-genome sequencing in routine diagnostic microbiology. Clin Microbiol Infect. 2018;24(4):355-60. Doi: 10.1016/j.cmi.2017.11.001. PMID: 29117578.

[9] CRyPTIC Consortium and the 100,000 Genomes Project, Allix-Béguec C, Arandjelovic I, Bi L, Beckert P, Bonnet M, et al. Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. N Engl J Med. 2018;379(15):1403-15. Doi: 10.1056/NEJMoa1800474. PMID: 30280646; PMCID: PMC6121966.

[10] Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. Antimicrobial resistance: A growing serious threat for global public health. Healthcare (Basel). 2023;11(13):1946. Doi: 10.3390/healthcare11131946. PMID: 37444780; PMCID: PMC10340576.

[11] Tang KWK, Millar BC, Moore JE. Antimicrobial resistance (AMR). Br J Biomed Sci. 2023;80:11387. Doi: 10.3389/bjbs.2023.11387. PMID: 37448857; PMCID: PMC10336207.

[12] Antimicrobial Resistance Division, National Action Plans and Monitoring and Evaluation. Global action plan on antimicrobial resistance. Geneva, Switzerland: World Health Organization; 2016:01-45. [cited 2024 Dec 25]. Available from: https://iris.who.int/bitstream/handle/10665/193736/9789241509763_eng.pdf?sequence=1.

[13] Govindaraj Vaithinathan A, Vanitha A. WHO global priority pathogens list on antibiotic resistance: An urgent need for action to integrate One Health data. Perspect Public Health. 2018;138(2):87-88. Doi: 10.1177/1757913917743881. PMID: 29465015.

[14] Comas I, Cancino-Muñoz I, Mariner-Llicer C, Goig GA, Ruiz-Hueso P, Francés-Cuesta C, et al. Use of next-generation sequencing technologies for the diagnosis and epidemiology of infectious diseases. Enferm Infecc Microbiol Clin (Engl Ed). 2020;38(Suppl 1):32-38. Doi: 10.1016/j.eimc.2020.02.006. PMID: 32111363.

[15] Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, et al. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. Genome Med. 2014;6(11):90. Doi: 10.1186/s13073-014-0090-6. PMID: 25422674; PMCID: PMC4237778.

[16] Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: Rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom. 2017;3(10):e000131. Doi: 10.1099/mgen.0.000131. PMID: 29177089; PMCID: PMC5695208.

[17] Mancuso G, Midiri A, De Gaetano S, Ponzo E, Biondo C. Tackling drug-resistant tuberculosis: New challenges from the old pathogen *Mycobacterium tuberculosis*. Microorganisms. 2023;11(9):2277. Doi: 10.3390/microorganisms11092277. PMID: 37764122; PMCID: PMC10537529.

[18] Holmes KK, Bertozzi S, Bloom BR, Jha P, (eds). Major infectious diseases. 3rd ed. Washington (DC): The International Bank for Reconstruction and Development/ The World Bank; 2017. Chapter 11. PMID: 30212055.

[19] Li T, Du X, Kang J, Luo D, Liu X, Zhao Y. Patient, diagnosis, and treatment delays among tuberculosis patients before and during the COVID-19 epidemic-China, 2018-2022. China CDC Wkly. 2023;5(12):259-65. Doi: 10.46234/ccdcw2023.047. PMID: 37138894; PMCID: PMC10150750.

[20] Dheda K, Perumal T, Moultrie H, Perumal R, Esmail A, Scott AJ, et al. The intersecting pandemics of tuberculosis and COVID-19: Population-level and patient-level impact, clinical presentation, and corrective interventions. Lancet Respir Med. 2022;10(6):603-22. Doi: 10.1016/S2213-2600(22)00092-3. PMID: 35338841; PMCID: PMC8942481.

[21] World Health Organization. Tuberculosis Deaths and Disease Increase during the COVID-19 Pandemic. Global Tuberculosis Report 2022. Geneva, Switzerland: WHO; 2022. [cited 2024 Dec 25]. Available from: https://www.who.int/news/item/27-10-2022-tuberculosis-deaths-and-disease-increase-during-the-covid-19-pandemic#:~:text=An%20estimated%2010.6%20million%20people,Organization's%202022%20Global%20TB%20report.

[22] Comas I. Genomic epidemiology of tuberculosis. Adv Exp Med Biol. 2017;1019:79-93. Doi: 10.1007/978-3-319-64371-7_4. PMID: 29116630.
[23] Wyllie DH, Davidson JA, Grace Smith E, Rathod P, Crook DW, Peto TEA, et al. A quantitative evaluation of MIRU-VNTR typing against whole-genome sequencing for identifying *Mycobacterium tuberculosis* transmission: A prospective observational cohort study. EBioMedicine. 2018;34:122-30. Doi: 10.1016/j.ebiom.2018.07.019. PMID: 30077721; PMCID: PMC6116353.
[24] Stucki D, Ballif M, Egger M, Furrer H, Altpeter E, Battegay M, et al. Standard genotyping overestimates transmission of Mycobacterium tuberculosis among immigrants in a low-incidence country. J Clin Microbiol. 2016;54(7):1862-70. Doi: 10.1128/JCM.00126-16. PMID: 27194683; PMCID: PMC4922098.
[25] Fiebig L, Kohl TA, Popovici O, Mühlenfeld M, Indra A, Homorodean D, et al. A joint cross-border investigation of a cluster of multidrug-resistant tuberculosis in Austria, Romania, and Germany in 2014 using classic, genotyping, and whole genome sequencing methods: Lessons learnt. Euro Surveill. 2017;22(2):30439. Doi: 10.2807/1560-7917.ES.2017.22.2.30439. PMID: 28106529; PMCID: PMC5404487.
[26] Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, et al. Whole genome sequencing of *Mycobacterium tuberculosis*: Current standards and open issues. Nat Rev Microbiol. 2019;17(9):533-45. Doi: 10.1038/s41579-019-0214-5. PMID: 31209399.
[27] Iwai H, Kato-Miyazawa M, Kirikae T, Miyoshi-Akiyama T. CASTB (the comprehensive analysis server for the *Mycobacterium tuberculosis* complex): A publicly accessible web server for epidemiological analyses, drug-resistance prediction, and phylogenetic comparison of clinical isolates. Tuberculosis (Edinb). 2015;95(6):843-44. Doi: 10.1016/j.tube.2015.09.002. PMID: 26542225.
[28] Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med. 2015;7(1):51. Doi: 10.1186/s13073-015-0164-0. PMID: 26019726; PMCID: PMC4446134.
[29] Feuerriegel S, Schleusener V, Beckert P, Kohl TA, Miotto P, Cirillo DM, et al. PhyResSE: A web tool delineating *Mycobacterium tuberculosis* antibiotic resistance and lineage from whole-genome sequencing data. J Clin Microbiol. 2015;53(6):1908-14. Doi: 10.1128/JCM.00025-15. PMID: 25854485; PMCID: PMC4432036.
[30] Gröschel MI, Walker TM, van der Werf TS, Lange C, Niemann S, Merker M. Pathogen-based precision medicine for drug-resistant tuberculosis. PLoS Pathog. 2018;14(10):e1007297. Doi: 10.1371/journal.ppat.1007297. PMID: 30335850; PMCID: PMC6193714.
[31] Robinson CM, Pfeiffer JK. Viruses and the microbiota. Annu Rev Virol. 2014;1:55-69. Doi: 10.1146/annurev-virology-031413-085550. PMID: 25821837; PMCID: PMC4373533.
[32] Jurasz H, Pawłowski T, Perlejewski K. Contamination issue in viral metagenomics: Problems, solutions, and clinical perspectives. Front Microbiol. 2021;12:745076. Doi: 10.3389/fmicb.2021.745076. PMID: 34745046; PMCID: PMC8564396.
[33] Doan T, Wilson MR, Crawford ED, Chow ED, Khan LM, Knopp KA, et al. Illuminating uveitis: Metagenomic deep sequencing identifies common and rare pathogens. Genome Med. 2016;8(1):90. Doi: 10.1186/s13073-016-0344-6. PMID: 27562436; PMCID: PMC4997733.
[34] Kawada J, Okuno Y, Torii Y, Okada R, Hayano S, Ando S, et al. Identification of viruses in cases of pediatric acute encephalitis and encephalopathy using next-generation sequencing. Sci Rep. 2016;6:33452. Doi: 10.1038/srep33452. PMID: 27625312; PMCID: PMC5022051.
[35] Mohamed S, Penaranda G, Gonzalez D, Camus C, Khiri H, Boulmé R, et al. Comparison of ultra-deep versus Sanger sequencing detection of minority mutations on the HIV-1 drug resistance interpretations after virological failure. AIDS. 2014;28(9):1315-24. Doi: 10.1097/QAD.0000000000000267. PMID: 24698843.
[36] Mbisa JL, Kirwan P, Tostevin A, Ledesma J, Bibby DF, Brown A, et al. Determining the origins of human immunodeficiency virus type 1 drug-resistant minority variants in people who are recently infected using phylogenetic reconstruction. Clin Infect Dis. 2019;69(7):1136-43. Doi: 10.1093/cid/ciy1048. PMID: 30534981; PMCID: PMC6743824.
[37] Abe H, Hayes CN, Hiraga N, Imamura M, Tsuge M, Miki D, et al. A translational study of resistance emergence using sequential direct-acting antiviral agents for hepatitis C using ultra-deep sequencing. Am J Gastroenterol. 2013;108(9):1464-72. Doi: 10.1038/ajg.2013.205. PMID: 23896953.
[38] Garrigue I, Moulinas R, Recordon-Pinson P, Delacour ML, Essig M, Kaminski H, et al. Contribution of next-generation sequencing to early detection of cytomegalovirus UL97 emerging mutants and viral subpopulations analysis in kidney transplant recipients. J Clin Virol. 2016;80:74-81. Doi: 10.1016/j.jcv.2016.04.017. PMID: 27214758.
[39] Lowe CF, Merrick L, Harrigan PR, Mazzulli T, Sherlock CH, Ritchie G. Implementation of next-generation sequencing for hepatitis B virus resistance testing and genotyping in a clinical microbiology laboratory. J Clin Microbiol. 2016;54(1):127-33. Doi: 10.1128/JCM.02229-15. PMID: 26537448; PMCID: PMC4702765.
[40] Watanabe S, Morimoto N, Miura K, Takaoka Y, Nomoto H, Tsukui M, et al. Full-genome characterization of the RIVM-HAV16-090-like hepatitis A virus strains recovered from Japanese men who have sex with men, with sporadic acute hepatitis A. Hepatol Res. 2019;49(5):521-30. Doi: 10.1111/hepr.13313. PMID: 30645783.
[41] Goldhill DH, Langat P, Xie H, Galiano M, Miah S, Kellam P, et al. Determining the mutation bias of favipiravir in influenza virus using next-generation sequencing. J Virol. 2019;93(2):e01217-18. Doi: 10.1128/JVI.01217-18. PMID: 30381482; PMCID: PMC6321902.
[42] Günthard HF, Calvez V, Paredes R, Pillay D, Shafer RW, Wensing AM, et al. Human immunodeficiency virus drug resistance: 2018 recommendations of the International Antiviral Society-USA Panel. Clin Infect Dis. 2019;68(2):177-87. Doi: 10.1093/cid/ciy463. PMID: 30052811; PMCID: PMC6321850.
[43] Cozzi-Lepri A, Noguera-Julian M, Di Giallonardo F, Schuurman R, Däumer M, Aitken S, et al. Low-frequency drug-resistant HIV-1 and risk of virological failure to first-line NNRTI-based ART: A multicohort European case-control study using centralized ultrasensitive 454 pyrosequencing. J Antimicrob Chemother. 2015;70(3):930-40. Doi: 10.1093/jac/dku426. PMID: 25336166; PMCID: PMC4319483.
[44] Metzner KJ, Rauch P, von Wyl V, Leemann C, Grube C, Kuster H, et al. Efficient suppression of minority drug-resistant HIV type 1 (HIV-1) variants present at primary HIV-1 infection by ritonavir-boosted protease inhibitor-containing antiretroviral therapy. J Infect Dis. 2010;201(7):1063-71. Doi: 10.1086/651136. PMID: 20196655.
[45] Brown RJ, Peters PJ, Caron C, Gonzalez-Perez MP, Stones L, Ankghuambom C, et al. Intercompartmental recombination of HIV-1 contributes to env intrahost diversity and modulates viral tropism and sensitivity to entry inhibitors. J Virol. 2011;85(12):6024-37. Doi: 10.1128/JVI.00131-11. PMID: 21471230; PMCID: PMC3126287.
[46] Pérez PS, Di Lello FA, Mullen EG, Galdame OA, Livellara BI, Gadano AC, et al. Compartmentalization of hepatitis C virus variants in patients with hepatocellular carcinoma. Mol Carcinog. 2017;56(2):371-80. Doi: 10.1002/mc.22500. PMID: 27163636.
[47] Caro-Pérez N, Martínez-Rebollar M, Gregori J, Quer J, González P, Gambato M, et al. Phylogenetic analysis of an epidemic outbreak of acute hepatitis C in HIV-infected patients by ultra-deep pyrosequencing. J Clin Virol. 2017;92:42-47. Doi: 10.1016/j.jcv.2017.05.008. PMID: 28521213.
[48] Zhou Z, Ma P, Feng Y, Ou W, Wei M, Shao Y. The inference of HIV-1 transmission direction between a man who has sex with men and his heterosexual wife based on the sequences of HIV-1 quasi-species. Emerg Microbes Infect. 2021;10(1):1209-16. Doi: 10.1080/22221751.2021.1938693. PMID: 34077305; PMCID: PMC8676586.
[49] Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science. 2014;345(6202):1369-72. Doi: 10.1126/science.1259657. PMID: 25214632; PMCID: PMC4431643.
[50] Faria NR, Quick J, Claro IM, Thézé J, de Jesus JG, Giovanetti M, et al. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature. 2017;546(7658):406-10. Doi: 10.1038/nature22401. PMID: 28538727; PMCID: PMC5722632.
[51] Stapleford KA, Moratorio G, Henningsson R, Chen R, Matheus S, Enfissi A, et al. Whole-genome sequencing analysis from the Chikungunya virus Caribbean outbreak reveals novel evolutionary genomic elements. PLoS Negl Trop Dis. 2016;10(1):e0004402. Doi: 10.1371/journal.pntd.0004402. PMID: 26807575; PMCID: PMC4726740.
[52] Fernández-Billón M, Llambías-Cabot AE, Jordana-Lluch E, Oliver A, Macià MD. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Biofilm. 2023;5:100129. Doi: 10.1016/j.bioflm.2023.100129. PMID: 37205903; PMCID: PMC10189392.
[53] Sherry NL, Porter JL, Seemann T, Watkins A, Stinear TP, Howden BP. Outbreak investigation using high-throughput genome sequencing within a diagnostic microbiology laboratory. J Clin Microbiol. 2013;51(5):1396-401. Doi: 10.1128/JCM.03332-12. PMID: 23408689; PMCID: PMC3647928.
[54] Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014;370(25):2408-17. Doi: 10.1056/NEJMoa1401268. PMID: 24896819; PMCID: PMC4134948.
[55] Mu A, Kwong JC, Isles NS, Gonçalves da Silva A, Schultz MB, Ballard SA, et al. Reconstruction of the genomes of drug-resistant pathogens for outbreak investigation through metagenomic sequencing. mSphere. 2019;4(1):e00529-18. Doi: 10.1128/mSphere.00529-18. PMID: 30651402; PMCID: PMC6336080.
[56] Casto AM, Adler AL, Makhsous N, Crawford K, Qin X, Kuypers JM, et al. Prospective, real-time metagenomic sequencing during norovirus outbreak reveals discrete transmission clusters. Clin Infect Dis. 2019;69(6):941-48. Doi: 10.1093/cid/ciy1020. PMID: 30576430; PMCID: PMC6735836.
[57] Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med. 2016;8(1):42. Doi: 10.1186/s13073-016-0303-2. PMID: 27098727; PMCID: PMC4839080.
[58] Haran JP, Bhattarai SK, Foley SE, Dutta P, Ward DV, Bucci V, et al. Alzheimer's disease microbiome is associated with dysregulation of the anti-inflammatory P-glycoprotein pathway. mBio. 2019;10(3):e00632-19. Doi: 10.1128/mBio.00632-19. PMID: 31064831; PMCID: PMC6509190.
[59] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013;368(5):407-15. Doi: 10.1056/NEJMoa1205037. PMID: 23323867.
[60] Besser J, Carleton HA, Gerner-Smidt P, Lindsey RL, Trees E. Next-generation sequencing technologies and their application to the study and control of bacterial infections. Clin Microbiol Infect. 2018;24(4):335-41. Doi: 10.1016/j.cmi.2017.10.013. PMID: 29074157; PMCID: PMC5857210.
[61] Jernigan DB, Raghunathan PL, Bell BP, Brechner R, Bresnitz EA, Butler JC, et al. Investigation of bioterrorism-related anthrax, United States, 2001: Epidemiologic findings. Emerg Infect Dis. 2002;8(10):1019-28. Doi: 10.3201/eid0810.020353. PMID: 12396909; PMCID: PMC2730292.

[62] Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T. Molecular subtyping of Bacillus anthracis and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis. 2002;8(10):1111-16. Doi: 10.3201/eid0810.020394. PMID: 12396925; PMCID: PMC2730295.

[63] Francés-Cuesta C, de la Caba I, Idigoras P, Fernández-Rodríguez A, Del Valle Pérez D, Marimón JM, et al. Whole-genome sequencing of Neisseria gonorrhoeae in a forensic transmission case. Forensic Sci Int Genet. 2019;42:141-46. Doi: 10.1016/j.fsigen.2019.07.003. PMID: 31319352.

PARTICULARS OF CONTRIBUTORS:
1. Student, Department of General Medicine, Maheshwara Medical College and Hospital, Hyderabad, Telangana, India.
2. Doctor, Department of Internal Medicine, Sri Laxmi Multi Speciality Hospital, Laproscopic and Research Centre, Hyderabad, Telangana, India.
3. Student, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.
4. Student, Department of General Medicine, SVS Medical College, Mahabubnagar, Telangana, India.
5. Doctor, Honorary International Faculty, AJ Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Yethindra Vityala,
2-5-168, Opposite Maruthi Towers, Nakkalgutta, Warangal-506001, Telangana, India.
E-mail: yethindravityala10@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 17, 2025
- Manual Googling: Jan 31, 2025
- iThenticate Software: Mar 03, 2025 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 5

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 15, 2025**
Date of Peer Review: **Jan 23, 2025**
Date of Acceptance: **Mar 05, 2025**
Date of Publishing: **Apr 01, 2025**