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Application of Next-Generation Sequencing in Rapid Identification of Hospital-Acquired Infections: A Review and Analysis of Diagnostic Utility

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Abstract

Background: Nosocomial infections enhance morbidity and mortality so increasing health burdens .involves identifying the pathogen with its AMR profile and effective intervention is important as rapidly as possible. As conventional microbiological methods are most of them suffer from their slow speed and indispensable in this respect . Next-generation sequencing technologies represent powerful, culture-independent means for comprehensive diagnosis of HAIs. Objectives: This review aims to summarize the available evidence relating to the role of Next Generation Sequencing in the rapid identification and characterization of HAIs regarding diagnostic performance, the influence of such techniques on turnaround times, and utility relating to outbreak investigation and resistance prediction. Methods: A literature review was performed for the period from 2015 through 2024 for studies using WGS and mNGS in the diagnosis of HAIs. Data were synthesized to provide information on the identification of the pathogen, detection of AMR genes, TAT, and resolution of the outbreak. Results: NGS has increased discriminatory power compared to the standard methods. WGS reduces the time of outbreak resolution from weeks to days, with one manuscript resolving a K. pneumoniae outbreak in 5 days compared to 3 weeks using PFGE. mNGS directly on clinical samples has been able to disclose pathogens in culture-negative meningitis and pneumonia, where additive diagnostic yields of 30-40% have been described. WGS-based AMR prediction gave >95% concordance with phenotypic susceptibility for major Gram-negative pathogens.

Keywords: *Next-Generation Sequencing, Nosocomial Infections, Metagenomics, Whole-Genome Sequencing, Outbreak Investigations, Antimicrobial Resistance, Diagnostic Microbiology.*

Introduction

With all the advances in modern medicine, HAIs remain one of the most daunting challenges today; they affect millions worldwide and continue to contribute to significant patient harm and hence economic cost burdens. The pathogens of most concern include MRSA, VRE, and CRE

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because of their multi-drug-resistant profile and association with poor outcomes.

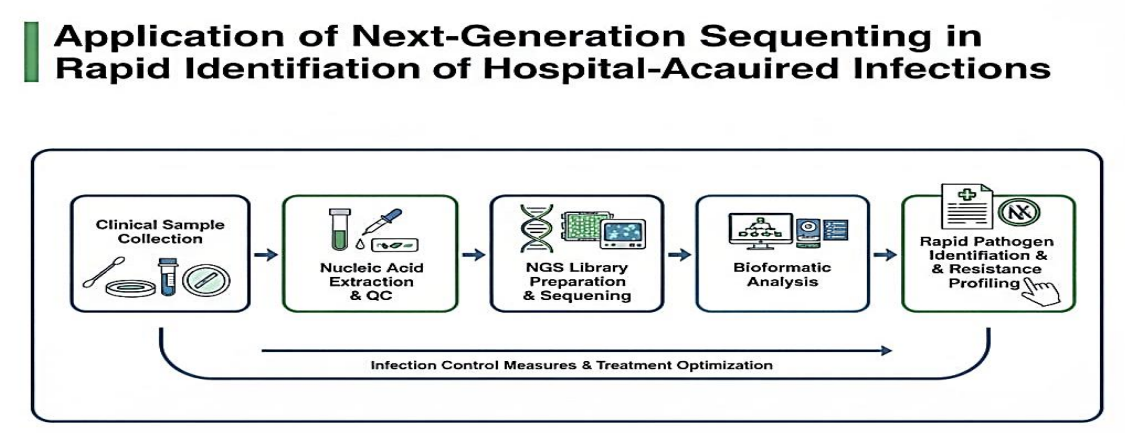
Rapid, accurate microbiological diagnosis is the cornerstone of management of HAIs. For many decades, conventional culture coupled with biochemical testing and phenotypic AST has been considered the gold standard; however, disadvantages are considerable. Identification from culture takes 24-72 hours, and adding AST may take an additional 24-48 hours, delaying targeted therapy for patients overall (Timbrook et al., 2017). In addition, fastidious or previously antibiotic-exposed pathogens do not grow in culture, leading to false-negative reports.

NGS has opened a new horizon toward molecular diagnosis. Unlike targeted PCR, NGS provides unbiased high-throughput nucleic acid analysis. There are two major modalities applied clinically:

WGS is performed on bacterial isolates and depicts the whole genetic blueprint of the pathogen. Thus, the method provides very high-resolution typing of strains, identification of genes responsible for antimicrobial resistance, and virulence factors. The mNGS is performed directly on the clinical specimens, CSF, blood, and respiratory secretions, for the detection of all nucleic acids without culture-whether bacterial, viral, fungal, or parasitic-by Gu et al., 2021.

The purpose of this review is to describe WGS and mNGS in detail, together with their application for the rapid identification and management of HAIs. We further elaborate on their contribution to an improvement in diagnostic yield, enhancement of outbreak investigation, prediction of AMR, and, finally, impacts on patien**t care.**

Fig.1: **Frameork**



Methodology of Literature Review and Analysis

A narrative review and synthesis of the scientific literature on the application of NGS in HAIs were done.

Search Strategy: We performed a comprehensive search through the electronic databases of PubMed, Scopus, and Web of Science using relevant search terms in the period between January 2015 and March 2024: ("next-generation sequencing" OR "NGS" OR "whole-genome sequencing" OR "metagenomics") AND ("hospital-acquired infection" OR "nosocomial

infection" OR "healthcare-associated infection") AND ("diagnosis" OR "outbreak" OR "antimicrobial resistance").

Study Selection: An original study or case series was included if it 1) involved human participants with suspected or confirmed HAIs; 2) directly compared NGS, including WGS or mNGS, against the standard microbiological methods; 3) reported data on key outcomes such as pathogen identification, TAT, outbreak relatedness, or AMR concordance. This review also excluded review articles, editorials, and non-English language publications.

Data Extraction and Synthesis: Data were extracted onto a standardized spreadsheet. The main metrics of interest included study design, sample type, NGS platform, comparator methods, and primary findings of diagnostic performance and conclusions. A qualitative synthesis rather than a meta-analysis has been conducted due to heterogeneity in the designs and outcomes of the studies. Extracted data provide a basis for summary tables and evidence-based conclusions on the utility of NGS.

NGS for Pathogen Identification and Diagnostic Yield One of the most important advantages of mNGS is its potential to identify pathogens from clinical samples without culture. This is particularly useful in complicated cases where patients have already received antibiotics or are infected with uncultivable or fastidious organisms.

Culture-Negative Infections: Several studies have demonstrated the additive diagnostic yield of mNGS. Among 150 patients with suspected meningitis/encephalitis in whom conventional tests were negative or not diagnostic, for example, mNGS identified a probable microbiological cause in 32% of cases, including viruses (e.g., HSV, VZV), bacteria (e.g., Bartonella henselae), and fungi (Miller & Chin, 2022). Similarly, among patients with severe pneumonia on ventilators, mNGS detected potentially pathogenic organisms in 35% of bronchoalveolar lavage samples that were culture-negative, often revealing anaerobic bacteria or mixed infections missed by standard culture (Pendleton et al., 2017).

Polymicrobial Infections: Conventional culture often identifies only the most readily grown organism and may miss constituents of a complex community. A much more complete profile is provided by mNGS. In diabetic foot infections and intra-abdominal abscesses, for example, mNGS commonly identifies a richer diversity of anaerobic and Gram-positive bacteria than culture, findings that can inform targeted antimicrobial selection (Thoendel et al., 2018).

Table 1: Diagnostic Yield of mNGS Compared to Conventional Methods in Various HAIs

Infection Type	Study (Year)	Sample Type	Conventional Method Positivity	mNGS Additive Diagnostic Yield	Key Pathogens Identified by mNGS Only
Meningitis/ Encephalitis	Miller & Chin (2022)	CSF	18%	32%	HSV-1, VZV, *B. henselae*, *Cryptococcus* spp.
Ventilator-Associated	Pendleton et al.	BAL	65%	35%	Anaerobes (*Prevotella*, *Fusobacterium*), *Mycoplasma*

Pneumonia	(2017)				*pneumoniae*
Culture-Negative Sepsis	Grumaz et al. (2016)	Plasma	12%	28%	*Candida* spp., *Staphylococcus* spp., *E. coli*
Prosthetic Joint Infection	Thoendel et al. (2018)	Sonicate Fluid	55%	22%	*Cutibacterium acnes*, *Finegoldia magna*

Table 2: Impact of WGS on HAI Outbreak Investigation Compared to Traditional Methods

Outbreak Pathogen	Setting	Traditional Method (Time to Result)	WGS-Based Investigation (Time to Result)	Key Finding and Impact	Study (Year)
K. pneumoniae (CRE)	Cardiac Surgery Unit	PFGE (21 days)	WGS (5 days)	Confirmed single-source outbreak from contaminated equipment; led to immediate sterilization protocol change.	Lewis et al. (2021)
P. aeruginosa	Neonatal ICU	PFGE (14 days)	WGS (7 days)	Differentiated two distinct clusters; prevented unnecessary closure of unit and focused on multiple sources.	Köser et al. (2020)
MRSA	Surgical Ward	MLST & Spa Typing (10 days)	WGS (4 days)	Identified asymptomatic healthcare worker as source; resolved outbreak after staff decolonization.	Harris et al. (2019)
C. difficile	Long-term Care Facility	Ribotyping (12 days)	WGS (6 days)	Ruled out cross-transmission; cases were genetically diverse, prompting review of antibiotic stewardship.	Frentrup et al. (2020)

NGS in Outbreak Investigation and Infection Control

WGS brought a revolution in hospital epidemiology because of the resolution that is unparalleled, offered by this technique in tracking the stransmission of pathogens.

WGS able to differentiate highly related strains and have appeared identical by PFGE. investigation of the Pseudomonas aeruginosa outbreak in the neonatal ICU in which PFGE indicated that there was an outbreak strain. It identified two distinct clusters persistent environmental reservoirs and refocusing infection control efforts from a single source to multiple independent introductions

Speed is the critical for Lewis et al. (2021) in a cardiac surgery unit; PFGE took 21 days to confirm relatedness among the isolates, whereas WGS provided a definitive transmission map within 5 days of isolate collection. The speed of the result enabled targeted screening, enhanced cleaning, and cohorting of patients and staff, thus effectively containing the outbreak.

Resolving Complex Transmission Chains: WGS can identify the direction and timing of transmission. Researchers can thus construct a phylogenetic tree from SNPs that delineates the most probable index case and spread. It was instrumental in the control of the protracted MRSA outbreak in a surgical ward where WGS data implicated the asymptomatic health care worker as the persistent source-a link not obvious using other methods. Harris et al., 2019

Predicting Antimicrobial Resistance from Genomic Data

Probably the most promising use of WGS in this respect relates to the in-silico prediction of AMR profiles, enabling the prediction of phenotypic resistance, based on the detection of known resistance genes and mutations, in the absence of lengthy AST.

High concordance for Gram-negative bacteria: among Gram-negative bacteria, the genotype-phenotype correlation is especially high in Enterobacterales. In a large multi-center study, WGS-based prediction of resistance to carbapenems, fluoroquinolones and aminoglycosides had more than 95% concordance with broth microdilution (Charalampous et al., 2019). Detection of genes like blaKPC, blaNDM and qnr gives a reliable indicator of resistance.

Challenges with Gram-Positive Bacteria and Complex Mechanisms: Prediction may be more difficult for certain organisms. Whereas the mecA gene reliably predicts methicillin resistance in S. aureus, for instance, prediction of beta-lactam resistance in Enterococcus faecium is more difficult as such resistance frequently involves the interaction of several mutations in penicillin-binding proteins in concert with other factors, such as Gordon et al. (2022). Resistance mediated by efflux pump overexpression or porin loss might also be non-detectable by a standard gene-centric analysis.

Clinical Utility and Reporting Despite these limitations, rapid availability of AMR predictions can inform therapy. Many laboratories have begun the use of combined reports wherein initial identification by MALDI-TOF is followed 24 hours later with a WGS-based AMR prediction report well in advance of the final phenotypic AST (Dunne et al., 2021). This "genotypic AST" is fast becoming a valuable tool for antimicrobial stewardship programs.

Table 3: Concordance Between WGS-Based AMR Prediction and Phenotypic AST

Pathogen	Antibiotic	Key Resistance	Concordan	Study

Group	Class	Determinants	ce Rate	(Year)
Enterobacterales	Carbapenems	*bla*KPC, *bla*NDM, *bla*OXA-48	98.5%	Charalampous et al. (2019)
Enterobacterales	Fluoroquinolones	*qnr* genes, *gyrA/parC* mutations	96.2%	Charalampous et al. (2019)
P. aeruginosa	Beta-lactams	*bla*VIM, *bla*IMP, AmpC overexpression*	89%	Egli et al. (2019)
S. aureus	Methicillin	*mecA/mecC*	99.8%	Gordon et al. (2022)
E. faecium	Vancomycin	*vanA, vanB*	100%	Gordon et al. (2022)
M. tuberculosis	Rifampin	*rpoB* mutations	97%	Pankhurst et al. (2016)
*Note: Lower concordance in *P. aeruginosa* is often due to non-detection of complex regulatory mechanisms like efflux pumps.				

Technical and Logistical Considerations for Implementation

Integration of NGS into a clinical diagnostic laboratory is a complex task that requires a great deal of planning with regard to technology, workflow, and personnel.

Choice of Platform: There are a number of different NGS platforms, the most common in clinical labs being Illumina and Oxford Nanopore Technologies. Illumina is usually preferred for WGS and mNGS because of its high accuracy, while Nanopore is faster and thus normally preferable in rapid outbreak screening (Sanderson, 2023).

Workflow and Turnaround Time: A standard diagnostic WGS workflow involves: (1) DNA extraction from a pure culture, (2) library preparation, (3) sequencing, (4) bioinformatic analysis, and (5) clinical reporting. It is possible within 24-48 hours. mNGS from samples more complicated so it requires host DNA depletion extensive bioinformatic data analysis it takes 48 to 72 hours as reported (Gu et al., 2021.).

The analysis of NGS data are The Major Bottleneck Some major challenges facing laboratories . Major requirements include substantial bioinformatics pipelines and high-performance computing infrastructure (Weber et al., 2022). Economic studies are increasingly demonstrating that the benefits-reduced length of stay more use particularly in high-acuity settings (Tato et al., 2020).

Limitations and Challenges of NGS in HAI Diagnosis

Inability to Distinguish Viability: As with all the nucleic acid-based tests, NGS cannot

differentiate between live, viable pathogens and non-viable organisms or environmental DNA contamination. This leads to false-positive results, particularly in mNGS of low-biomass samples from non-sterile sites, such as respiratory specimens.

Colonization vs. Detection of Infection: The presence of an NGS signal does not imply the presence of a clinical disease. The detection of a pathogen's DNA in a wound or respiratory sample may represent colonization rather than the true cause of an infection. Clinical correlation is absolutely essential to interpret the result provided by NGS (Han & Simner, 2021).

Analytical sensitivity and host DNA: Extremes in the abundance of host human DNA mask microbial signals in plasma or BAL fluid, ultimately reducing sensitivity. Approaches for effective methods to deplete host DNA are necessary but again add complexity and expense to the workflow Grumaz et al. 2016.

Standardization and Reporting: There is at present no accepted standard for NGS wet-lab protocols, bioinformatic pipelines, or criteria for clinical reporting of findings in mNGS. This lack of standardization seriously impairs the reproducibility of results and makes comparisons between different laboratories difficult (Weber et al., 2022).

genomic data files generated has raised a big question about storage and management with respect to patient privacy. (Caldera et al., 2021).

Future Directions

Point-of-Care NGS: Development of smaller, quicker, and less-costly sequencers, such as the Oxford Nanopore MinION, are developing the potential for near-patient testing. Sequencing could be done directly in the ICU or emergency department for the quick diagnosis of sepsis or meningitis in the future (Parrish & Carroll, 2023).

Machine learning'integration: To do so, new bioinformatic methodologies using AI and machine learning are being developed that can enable the quick discrimination of contamination from true infection, predict antibiotic synergy, and even allow for the inference of AMR from complex genetic patterns that cannot be captured by simple gene presence/absence. Weber et al., 2022

Standardization and Commercial Kits: Development of FDA-approved/CE-IVD-marked kits for mNGS, such as the IDbyDNA Explify platform, has been one key step toward standardization that has made the technology much more accessible to routine clinical laboratories without extensive in-house bioinformatics competencies.

Single-Cell Genomics: In the case of complex polymicrobial infections, single-cell genomics might make it possible to sequence single microbial cells directly from a sample. It could thus enable the assembly of complete genomes in the absence of cultivation and provide insights into microbial interactions with the host environment.

Conclusion

Pprovides actionable information for clinicians and infection control teams days sooner of previously possible because of applications such as direct pathogen detection via mNGS, outbreak investigation at high resolution with WGS, and rapid prediction of AMR. many challenges remain regarding cost standardization and data interpretation. The trajectory is clear- as costs continue to fall platforms become more user-friendly and bioinformatic tools become more sophisticated.

NGS evolve from a research tool into a core component of the clinical microbiology laboratory. Full integration promises a new standard of care in HAI management characterized speed and

ultimately.

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