Rapid high-throughput sequencing: a game-changer for timely addressing infectious diseases

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Infectious diseases, particularly bloodstream infections (BSIs) and sepsis, remain significant contributors to global morbidity and mortality. Traditional blood culture and antimicrobial susceptibility testing are time-consuming, often delaying optimal treatments. Targeted molecular diagnostic techniques, such as polymerase chain reaction (PCR), are limited in their coverage of pathogens and resistance markers, failing to provide comprehensive genomic information. Although clinical metagenomics based on short-read sequencing has gradually been integrated into clinical practice, most established workflows still require nearly 24 h or longer turnaround times (TAT) to complete testing.[1–3] Moreover, sequencing data often lack sufficient gene coverage to accurately predict pathogen resistance and other functional characteristics.[4] In recent years, research conducted by our team and others has preliminarily demonstrated the significant potential of real-time sequencing to identify pathogens and predict resistance within a few hours (5–7 h),[5–7] offering new possibilities to address complex infections in clinical settings more rapidly.

Notably, in a recent paper published in *eBioMedicine*,[8] Di Pilato and colleagues developed an innovative workflow (termed LC-WGS) for the rapid identification of bacteria and their resistance profiles from positive blood culture (BC) bottles. This workflow integrates a commercial system for rapid purification of microbial cells from positive blood cultures (Qvella FAST System, Qvella), which enables the removal of free DNA and host DNA, with a real-time sequencing platform (MinION, Oxford Nanopore Technologies) and a streamlined bioinformatics pipeline. This combination enables pathogen identification, resistance gene profiling, and bacterial subtyping within a short timeframe. Validation using 85 prospectively collected positive blood culture samples demonstrated that the workflow could accurately identify bacterial pathogens in approximately 2.6 h and detect all major clinically relevant resistance genes (including variant-level detection) within 4 h. This timeframe is significantly shorter than that of traditional BC workflows. By rapidly identifying pathogens and their resistance profiles, this technology is expected to correct empirical treatment biases, optimise antibiotic selection, and provide critical decision-making support for sepsis and other severe infections, thereby potentially improving patient outcomes and reducing healthcare risks. The study also highlighted LC-WGS's effectiveness in managing polymicrobial infections. While traditional blood cultures struggle with polymicrobial infections, LC-WGS, through high-throughput sequencing and bioinformatics analysis, can effectively distinguish among different pathogens and their resistance genes. This capability is particularly valuable in complex infection cases, enabling clinicians to devise more precise treatment strategies. Furthermore, the study demonstrated that LC-WGS-generated sequence data could support genomic surveillance initiatives, such as detecting virulence genes in extraintestinal pathogenic *E. coli* (ExPEC) and hypervirulent *Klebsiella pneumoniae*, serotyping *Neisseria meningitidis*, and enabling real-time comparative genomic analysis (e.g., tracking an NDM-producing ST147 *K. pneumoniae* outbreak in Tuscany, Italy). These applications underscore its potential value in public health and infection prevention and control.

The emergence of LC-WGS has opened new possibilities for the rapid diagnosis and treatment of bloodstream infections. Although the technology still relies on culture-positive samples, it can provide actionable clinical microbiological data from positive blood cultures within approximately 2.6–4 h, marking a significant step towards the routine application of rapid high-throughput sequencing in clinical microbiology laboratories. However, several technical challenges must be addressed for its broader clinical adoption, such as improving sequencing accuracy and sensitivity, particularly for pathogens with low microbial load, and optimising data analysis pipelines to translate sequencing results into clinically actionable information more efficiently. Addressing these challenges will require interdisciplinary collaboration, integrating advances in microbiology, clinical medicine, genomics, and bioinformatics. Looking ahead, it is worth exploring whether this approach can be further refined to enable direct identification of a broader range of pathogens (including bacteria, fungi, viruses, and parasites) from clinical

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samples, along with accurate characterisation of resistance, virulence, and epidemiological information. Additionally, reducing sequencing costs and simplifying workflows to make rapid high-throughput sequencing accessible in resource-limited settings will be a key focus for future research.

In conclusion, we anticipate that rapid diagnostic solutions, exemplified by real-time high-throughput sequencing technologies, will be increasingly integrated into infectious disease diagnostic pathways. These innovations, complementing traditional microbiological methods, will offer flexible applications across diverse clinical scenarios, ultimately delivering more efficient and precise diagnostic and therapeutic services to patients with infectious diseases.

Contributors

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Declaration of interests

The authors declare no conflict of interest.

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