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INTEGRATING RAPID DIAGNOSTICS, AI TECHNOLOGIES, AND OMICS APPROACHES FOR SEPSIS MANAGEMENT AND ANTIMICROBIAL STEWARDSHIP RUNNING TITLE: ADVANCES IN MICROBIOLOGY PATHOLOGY

Kaneez Fatima[1], Hiba Haroon[2], Ifra Laraib[3], Esha Jabbar[4], Fatima Yousaf[5], Iqra Jamil[6]

[1]Student, Department of Medical Laboratory Technology, Department of Microbiology, University of Central Punjab, Lahore, Email: kf747848@gmail.com
[2]Student, Department of Medical Laboratory Technology, Department of Microbiology, University of Central Punjab, Lahore, Email: hibajut127@gmail.com
[3]Student, Department of Medical Laboratory Technology, Department of Microbiology, University of Central Punjab, Lahore, Email: Ifralaraib30@gmail.com
[4]Student, Department of Medical Laboratory Technology, Department of Microbiology, University of Central Punjab, Lahore, Email: eshajabbar128@gmail.com
[5]Student, Department of Medical Laboratory Technology, Department of Microbiology, University of Central Punjab, Lahore, Email: Fatiyousuf0317@gmail.com
[6]Lecturer, Department of Microbiology, University of Central Punjab, Lahore
Email: iqrajameel@hotmail.com

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Corresponding Author: Iqra Jamil, Lecturer, Department of Microbiology, University of Central Punjab, Lahore
Email: iqrajameel@hotmail.com

ABSTRACT

The most recent developments in microbiological diagnostics are highlighted in this review, with an emphasis on quick and precise techniques that are essential for treating critically sick patients who may have bacterial infections. Rapid, accurate diagnostic tools that go beyond conventional culture-based methods are desperately needed, especially in light of the growing problem of antibiotic resistance and the high morbidity and mortality linked to sepsis in intensive care units. Improved pathogen identification and resistance profiling are promised by emerging technologies such as sophisticated omics techniques, nucleic acid amplification, direct-from-blood testing, and quick antigen detection. Diagnostic speed and accuracy are being revolutionized by AI-enhanced techniques like SepsetER and the Sepsis ImmunoScore, as well as cutting-edge platforms like T2 magnetic resonance and nanopore sequencing. However, these techniques still depends on their incorporation into strong antimicrobial stewardship initiatives

	and validation through well planned clinical trials. The potential of these new technologies is rigorously evaluated in this review, along with the crucial actions needed to turn them into improved patient outcomes.

Introduction

Bacterial diseases such as sepsis make up 37% and it affects 4% of ICU patients in Europe (24.7% on admission) and expresses high mortality, morbidity, and healthcare-related costs [1]. It emerges that about 70% of ICU patients worldwide require antibiotics every day. Fast detection is important, especially with high levels of resistance to antimicrobial drugs [2]. There is a need for diagnostic tools that can distinguish between infective and non-infective inflammation and encourage reduced utilization of antibiotics [3]. Current diagnostic strategies in sepsis usually include the **blood culture** to check for bacteraemia, but this strategy is time-consuming and is associated with pre-analytical sampling issues like, insufficient blood sample, previous antibiotic administration and time taken to deliver the sample to an off-site laboratory [4-6]. However, novel approaches such as matrix-assisted laser desorption/ionisation time of flight mass spectrometry (**MALDI-TOF**) provides rapid, inexpensive bacterial identification that can replace turn-around times (TATs) in microbiological diagnostics [7]. This article aims to identify new and developing technologies helping enhance rapid and accurate microbiological diagnosis in patients with severe **bacterial infections and sepsis** [8-9].

Established Rapid Diagnostic Methods

Developments in the rapid diagnostic tools and the automation of work flow systems have improved the healthcare at a great deal [10-13]. Current methods in culture media include automated blood culture (BC) systems such as BACTECTM FX and BacT/ALERT® for detection of organization growth in cases of bloodstream infections [14] . For example, the BacT/ALERT® VIRTUO system provides shorter time to detection and greater bacterial recovery [15-17]. These systems are generally based on internal sensing, that detect the microbial growth via carbon dioxide or change in pH [18] . Microscopy and Gram staining of sterile fluids should always be performed in severe infections, although these procedures are more time-consuming and depend on the skills of the operator [19].

SepsetER Test: Septicemia is a potentially fatal bloodstream infection that is a major global public health concern that can be fatal if left untreated with the right antimicrobial treatments [20]. The SepsetER test is a blood-based **gene expression** assay that employs AI to rapidly identify infections at increased risk of severe sepsis [21-22]. Developed by ASEP Medical Holdings Inc., this test provides results within about an **hour**, enabling swift risk assessment and intervention [23].

AI-Based Antimicrobial Susceptibility Testing: The Keynome gAST technology analyzes bacterial genomes directly from patient blood samples using machine learning methods [24]. By avoiding conventional culture techniques, this method makes it possible to anticipate antibiotic resistance quickly and accurately, which is essential for the prompt and efficient treatment of sepsis [25-26].

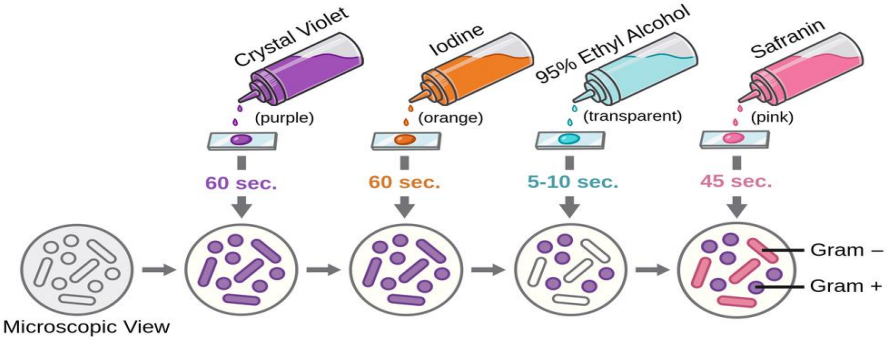


Figure 1: Workflow of gram staining method [27].

While machine learning in automated Gram stain picture acquisition and automated staining result classification are significant advancements, full auto-mode operation is still a long way off [28]. Because the coagulase test takes less time than previous procedures, it is now more feasible to identify pathogens like Staphylococcus aureus from BC [29-30]. Faster antimicrobial susceptibility testing, such as that developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), produces results in 4–8 hours [31].

β-lactamases: Commercial kits for the detection of resistance mediators include β-lactamases, which enable the quicker turnaround time of carbapenemase and extended-spectrum β-lactamase (ESBL) generating organisms than traditional approaches [32-33].

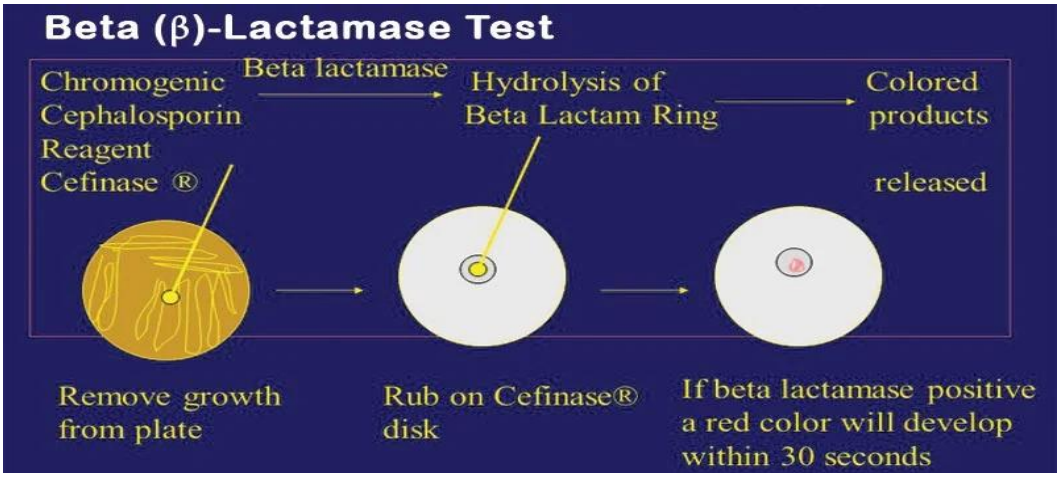


Figure 2: Beta (β) Lactamase Test [34].

The urine antigen test for *Streptococcus pneumoniae* and *L. pneumophila* in respiratory infections is one example of a serological test that uses direct antigens in clinical samples [35]. Nevertheless, these tests typically lack patterns of antibiotic resistance and have low sensitivity and specificity [36].

Sepsis ImmunoScore

The Sepsis ImmunoScore, an AI-powered diagnostic tool created by Prenosis, assesses 22 health indicators, such as blood pressure and vital signs, to produce a sepsis risk score [37]. This technique facilitates infection diagnosis and forecasts the probability of a severe sepsis developing within 24 hours, enabling timely action [38].

PCR or Nucleic acid amplification tests (NAAT)

Tests for nucleic acid amplification, or PCR, are non-culture techniques used to identify several diseases [39]. In contrast to single PCR, cold multiplex PCR amplifies the various specimens; however, it is technically complex, requires specialized facilities and skilled personnel, lacks antibiotic susceptibility data, and may not detect uncommon pathogens or low prevalent organisms [40–43]. Via inflammatory and anti-inflammatory modulators, sepsis and severe infection have been shown to trigger an immune response [44-45].

C-Reactive Protein (CRP)

The liver produces CRP, an acute-phase protein, in reaction to inflammation, which is mostly brought on by bacterial infections, trauma, or autoimmune disorders [46].

- In response to bacterial infections, levels increase quickly (within 6–12 hours).
- Bacterial infections are more likely to be indicated by higher CRP values (>100 mg/L) than viral infections [47].
- It is not exclusive to bacterial infections; it can also arise as a result of tissue damage, virus infections, or long-term inflammatory diseases such rheumatoid arthritis [48].

Procalcitonin (PCT)

PCT is a precursor of the hormone calcitonin, which is generated in reaction to bacterial infections but is not markedly increased in autoimmune disorders or viral infections [49].

- Extremely specific for bacterial infections, particularly those that are severe (meningitis, pneumonia, and sepsis) [50].
- Increases 2–6 hours after infection and peaks 24–48 hours later.
- While low levels (<0.1 ng/mL) suggest a viral infection or non-infectious inflammation, higher levels (>0.5 ng/mL) suggest a bacterial infection [51].
- Helpful in evaluating the efficacy of antibiotic therapy and in differentiating between bacterial and viral infections [52].

These biomarkers make it possible to manage antibiotic prescriptions and determine the patients' risk of infection. Among these is procalcitonin, which has been used to determine when to cease antibiotics and has reduced treatment time in intensive care units for both adults and newborns.

New and Emerging Methods

New rapid diagnostic tests (RDTs) can quickly identify pathogens and resistance profiles, with potential to improve patient management. However, studies on their clinical impact remain limited [54].

AI-Enhanced Imaging for Respiratory Diseases

By examining lung ultrasonography videos, an artificial intelligence algorithm developed by Charles Darwin University researchers can identify illnesses including pneumonia and COVID-19 [55]. The algorithm examines each video frame to identify significant lung features and patterns, with a diagnosis accuracy of 96.57% [56–57]. This method helps radiologists make clinical decisions by expediting diagnosis and producing results that can be explained [58].

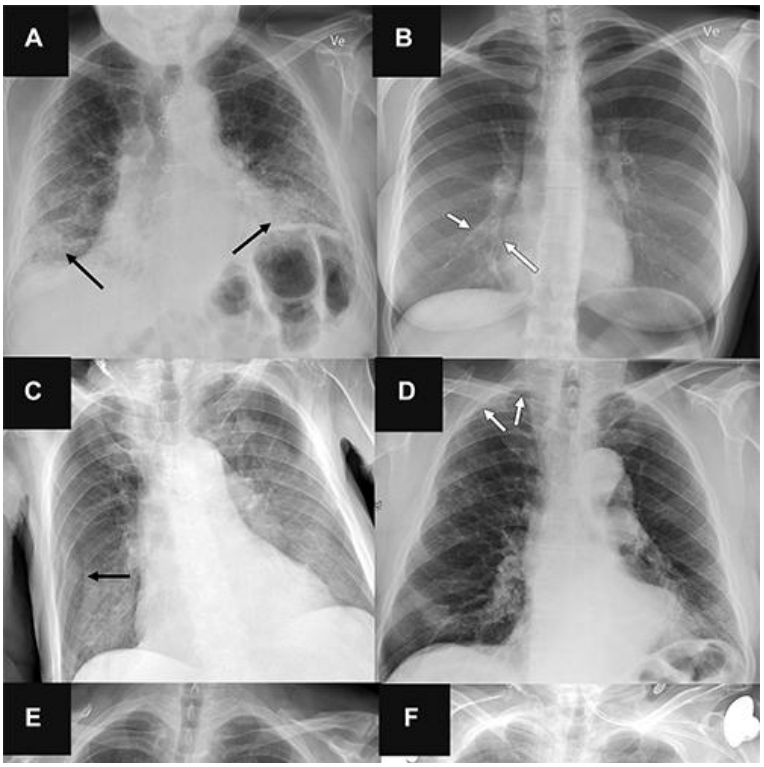
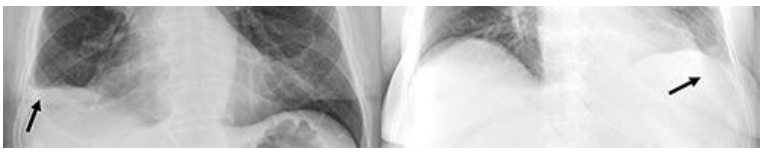


Figure 3: Representative chest radiographs in six patients show (A, C, E) false-positive findings and (B, D, F) false-negative findings as identified by the artificial intelligence (AI) tools [59].



New Approaches towards Infection Diagnosis

In ICU settings, multiplex PCR is being utilized more and more in clinical practice to diagnose pneumonia and infections of the central nervous system [60–61]. In just one hour, the BioFire FilmArray Meningitis/Encephalitis panel can identify 14 pathogens with 90–97% sensitivity and specificity [62]. In critically ill patients, the BioFire FilmArray Pneumonia plus Panel improves antibiotic stewardship by detecting 27 bacteria and 7 resistance indicators [63].

Personalized Treatment Plans for Respiratory Conditions

RhinoMAP is an AI-based tool being developed by Diag-Nose.io to customize treatment for respiratory diseases such as asthma and chronic obstructive pulmonary disease [64]. Within 48 hours, the AI suggests the best medication regimens based on biological data from nasal liquid biopsies, with the goal of symptom relief and better patient outcomes [65].

Nucleic Acid Detection from Blood Cultures

Several new rapid diagnostic tests can directly detect pathogens and resistance markers from positive blood cultures, without waiting for bacterial growth [66]. Examples include multiplex PCR panels like the BioFire FilmArray BC test, which identifies 24 pathogens and 3 resistance genes, and the **Xpert MRSA/SA BC assay**, which uses real-time PCR to detect methicillin-resistant and susceptible *Staphylococcus aureus* [67].

VERIGENE® Blood Culture Nucleic Acid Tests

With great sensitivity and specificity, these techniques enable the direct identification of bacteria and genetic resistance indicators from positive blood cultures [68].

22 microorganisms and their resistance determinants are identified from positive cultures using the Verigene technology, which uses multiplex PCR and microarray [69–70]. Another method is fluorescent in situ hybridization (FISH) using DNA probes that target bacterial and fungal ribosomal RNA [71]. Compared to conventional culture-based techniques, these quick assays may allow for quicker optimization of antibiotic medication [72].

Nanopore Sequencing Technology

Without requiring a previous culture, nanopore sequencing provides real-time analysis of microbial DNA straight from positive blood cultures, facilitating thorough pathogen identification and antibiotic resistance prediction [73].

Pathogen Detection Direct from Blood

New rapid diagnostic technologies can detect pathogens directly from whole blood samples, without the need for blood culture. This includes PCR-based tests like **SeptiFast** and **Magicplex**, which can identify multiple microbes and resistance markers, but have limited sensitivity [74]. The **T2 magnetic resonance** (T2MR) technology combines PCR with magnetic nanoparticles to quickly

identify common Candida species and **ESKAPE** bacteria from whole blood [75]. MALDI-TOF mass spectrometry and combined PCR/mass spectrometry platforms like **IRIDICA** also show promise for direct pathogen detection from clinical samples [76].

IRIDICA was a rapid molecular diagnostic platform developed by **Abbott** that used PCR/electrospray ionization mass spectrometry (PCR/ESI-MS) technology to detect bacterial, fungal, and viral pathogens directly from clinical specimens, including blood, respiratory, and sterile fluids, without requiring bacterial culture [77].

While these direct-from-blood methods have potential to improve antimicrobial stewardship, their clinical benefits are still uncertain, and some assays have been discontinued. Integrating these new diagnostics into effective antimicrobial stewardship programs remains a key challenge [78].

Direct Metagenomics

In clinical microbiology Metagenomics-based assays are the most important tools because they can find any type of microorganisms in a given sample [79].

16S metagenomics is based on amplifying the bacterial 16S rRNA gene using universal primers, followed by amplicon sequencing to identify bacteria and perform taxonomic profiling [80].

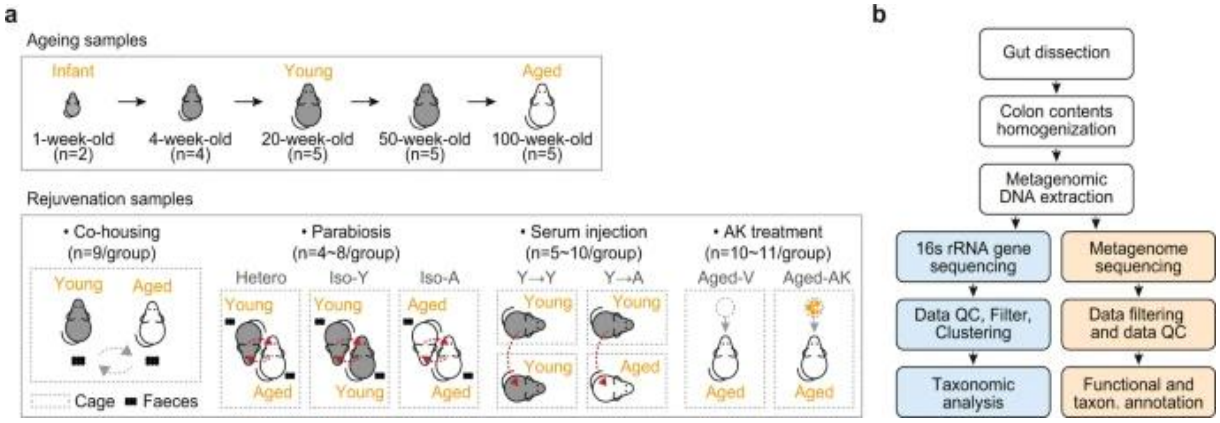


Figure 4: Overview of the experimental design. (a) Schematic description of the ageing model and four rejuvenation experiments including co-housing, parabiosis, serum injection, and AK treatment. (b) Workflow of data analysis. The analysis of 16 S rRNA data and metagenomic data are shown at the blue and yellow panel, respectively.

SepsiTest (Molzym) is used to detect pathogen in blood but it can't detect polymicrobial infections and fastidious organisms and doesn't provide AST. Moreover, its role in informing a clinical discussion is limited [81]. Shotgun metagenomics can read complete bacterial genomes by using parallel sequencing, as a result it can provide exact taxonomic resolution and detect markers of antimicrobial resistance of all pathogens. **iDTECT Dx** Blood (PathoQuest) can detect more clinically relevant microorganisms than conventional microbiology in immunocompromised patients, and its value of prediction is negative [82].

Karius NGS Plasma Test

From a single blood draw (plasma), the Karius Test, an AI-powered next-generation sequencing (NGS)-based metagenomic assay, can identify more than 1,000 pathogens (bacteria, fungus, DNA viruses, and parasites) [83]. It is a quick, non-invasive, culture-free diagnostic method for detecting bloodstream infections in critically ill and immunocompromised individuals [84]. The Karius Test is 93.7% more sensitive than BCs in patients and can identify microbial cell-free DNA from more than 1200 bacteria [85].

Although shotgun-metagenomics may fully analyze the microbial genetic material present in a sample, these procedures are challenging due to several constraints [86].

Virus Identification

Viral sequences in metagenomic data have been found using artificial intelligence. Tools like DeepVirFinder, which beat conventional methods in accuracy and speed, use deep learning algorithms to predict viral sequences [87].

Clear results for bloodstream infections may be difficult to obtain when contaminants and colonizers are detected during NGS testing. According to a recent study, the sepsis indicating quantifier (SIQ) is useful for differentiating infections that are clinically important [88]. Furthermore, NGS sensitivity is reduced in samples with a strong nucleic acid background, necessitating the reduction of human DNA [89]. Delays, extra expenses, and problems with data storage, privacy, and regulatory accreditation might result from labs lacking standardized analysis techniques and bioinformatics expertise [90].

New Rapid AST Methods

Detecting resistance genes may not always reflect the actual sensitivity pattern of the identified pathogen. The FDA-approved **Accelerate Pheno system** can detect 16 microorganisms and perform phenotypic AST with over 96% agreement compared to standard methods. Studies have shown that the time of optimal therapy in patients improves by this test [91].

AI-Based AST Methods

Name of Method	Time to Outcomes	Technology	Important Features
Oxford AI-Powered AST	~30 minutes	Machine Learning on bacterial growth	Fast, high accuracy, suitable for urgent care settings
GPT-4	~3-6 hours	AI (GPT-4) and	Detects resistance

Antimicrobial Resistance Detection	Real-time	genomic/phenotypic data [92]	mechanisms from genomic sequencing
Automated Optical System	~6 hours	Intensity & Deep Learning	Cost-effective, reduces incubation time
AI for Sepsis Diagnosis [93]	~12 hours	AI & Pathogen/Resistance Detection	Fast sepsis diagnosis, helps with early antibiotic choice [94]
Deep Learning Single-Cell	~3-6 hours	Deep Learning & Imaging	High sensitivity, structural changes at the cell level

Advances in **microfluidics, electronics, optics, and biosensor** techniques show promise for next-generation rapid AST. However, studies are ongoing to achieve FDA approval and CE marks because of their role in addressing point-of-care testing (POCT) needs is still scarce [95].

Transcriptomics

Current infection biomarkers provide limited insights into the host response to infection and offer only binary outcomes for infection severity or bacterial probability. More comprehensive infection characterization can be achieved through omics technologies, including **proteomics, metabolomics, epigenomics**, and transcriptomics [96]. Transcript-based diagnostics, such as the FDA-approved Septicyte, have the potential to differentiate between bacterial, viral or fungal pathogens and inflammatory phenotypes, potentially enabling personalized treatment for sepsis [97]. However, their implementation in ICU settings requires timely assay performance and demonstration of clinical and cost-effectiveness through trials. These advanced diagnostic tools can provide a more nuanced understanding of the host's response to infection, moving beyond the limitations of current biomarkers and enabling more targeted and effective treatment strategies [98].

Point of Care Diagnostics (POCT)

The use of laboratory-based tests can introduce delays and distance between the patient and the clinician, which can be particularly problematic in remote or resource-limited locations. In Australia and other regions, point of care testing (POCT) must adhere to strict governance and quality standards [99]. Unfortunately, at present, there are no POCT solutions that can accurately diagnose

bloodstream or critical infections. Future advances may include microfluidic devices that handle all molecular detection steps within a portable device. Despite substantial pre-clinical research, no commercial products are ready for clinical evaluation [100].

Evaluating Novel Rapid Diagnostics

Evaluating rapid diagnostic technologies involves more than just reduced turnaround time. Key factors include sensitivity, specificity, result type, and clinical confidence. Comprehensive evaluation should include trials or interrupted time series analyses measuring clinical and process outcomes, along with cost-effectiveness analyses [107]. However, high quality evidence in this area is sparce. Rapid technologies alone do not improve outcomes without integrated antimicrobial stewardship (AMS) strategies. Successful AMS strategies coupled with rapid diagnostics can improve antimicrobial use and de-escalation through the impact on clinical outcomes varies [108].

Barriers to effective implementation include prescribing behaviors and familiarity (RDT) results. For molecular RDTs to be successfully implemented, significant resources, ongoing phenotyping testing, and robust AMS support are required, tailored to local contexts [109].

Conclusion

AI-driven developments in pathology and microbiology are drastically changing research and diagnosis procedures. More efficiency, accuracy, and speed are provided by these advancements, which range from improving image analysis for histopathology to increasing pathogen identification and antibiotic resistance prediction. Methods like deep learning models for microbial resistance, AI-powered picture segmentation, and AI-enhanced DNA extraction are transforming the way researchers and doctors approach cancer pathology and microbiological diagnostics.

There is a chance that certain novel microbiological methods could enhance our capacity to promptly and precisely identify pathogens in patients in critical condition. However, before we can state with certainty more about these technologies and how effective they are at treating serious illnesses, we must do well structured study.

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