ISSN No. 2456-4400
Int J Med Lab Res 2025, 10(2), 34-42

IJMLR International Journal of Medical Laboratory Research

REVIEW ARTICLE

BIOCHEMICAL AND MOLECULAR APPROACHES TO BACTERIAL PATHOGEN IDENTIFICATION: TRADITIONAL TECHNIQUES AND EMERGING ADVANCES

Subhajit Karmakar[1], Athar Rehan[2], Rukshar Jahan[3], Mahenoor[4]

[1] *Lecturer, Mata Gujri College of Allied Health & Paramedical Sciences, Kishanganj, Bihar, India, 855107.*
[2,3,4] *Student, Mata Gujri College of Allied Health & Paramedical Sciences, Kishanganj, Bihar, India, 855107*

Received: 05 June, 2025 /Revision: 10 June, 2025 /Accepted: 20 June, 2025

ABSTRACT: In clinical microbiology, monitoring the environment and protecting food safety, having the ability to identify bacteria is critical for learning about infectious diseases, the growth of microbes and pathogens found in food. Tests that rely on bacteria's metabolic processes are used for telling apart and recognizing different kinds of bacteria. I will explain traditional and modern biochemical tests, what they involve, their strengths and their weaknesses. It looks at how microbiology is applied to clinical diagnostics, environmental monitoring and the food and drinks industry, handling problems related to using phenotypic methods and explaining recent developments that improve both. The review points out that reliability, reproducibility and similarity between test results from different laboratories depend on strict quality control and set protocols. Thanks to this, ribosomal proteins are still important in diagnosing with molecular methods and add to our understanding of bacteria and their social interactions

Keywords: Clinical microbiology, Infectious diseases, Metabolic processes, Biochemical tests, Clinical diagnostics, Phenotypic methods, Quality control, Molecular methods.

INTRODUCTION:

Being able to recognize bacterial diseases fast allows medical care for patients and helps protect the public. It is frequently incorrect to treat infection with antibiotics before knowing the cause, since it causes incorrect treatment, can increase resistance and can result in bad outcomes for patients [1]. Knowing what kind of bacteria there is allows for proper antibiotic use, controlling infections and following the spread of outbreaks [2].

Labs in clinical microbiology find bacterial pathogens using standard culture techniques and chemical analysis. Today's biotechnology involves running fermentation using carbohydrates, making enzymes and looking at the byproducts of biological processes. Biochemical tests are still very important because they are affordable, easy to use and provide useful knowledge from the molecule's present [3].

Corresponding Author:
Mr. Subhajit Karmakar, Lecturer,
Mata Gujri College of Allied Health & Paramedical Sciences, Kishanganj, Bihar, India, 855107
Email- subhajit.mgcoahps@gmail.com

Now that antimicrobial resistance in Gram-negative bacteria is starting to happen very rapidly, doctors need better methods to detect it in the laboratory [4]. Using advanced systems has made testing faster, analysis of data in real-time possible and made it simpler to monitor from a distance and distribute the results more quickly [5]. Using these new techniques, it is now much simpler to find out how microbes resist antibiotics and clinical researchers can study the causes of antimicrobial resistance [6]. Because of modern bioinformatics, the identification of resistance genes and the study of how they work happens quickly with detailed genomic studies.

1. PRINCIPLES OF BIOCHEMICAL TESTS

Biochemical tests identify bacteria's unique enzymatic and metabolic capabilities, detecting changes like color change, gas production, or pH alteration, allowing differentiation between them.

a. **Carbohydrate Fermentation:** A lot of bacteria can take carbohydrates such as glucose, lactose or sucrose and release acids, gases or alcohols as a result.

b. **Enzyme Activity:** These tests can detect enzymes like Catalase, Oxidase, Urease and Coagulase which are made by several bacteria.

c. **Metabolic Byproducts:** Many bacteria release chemicals called Indole, Hydrogen Sulfide and Acetoin that can be noticed with chemical tests and indicators.

2. METHODOLOGIES OF BIOCHEMICAL TESTS

Standard procedures and reagents are used for Biochemical Tests which helps all laboratory results to be consistent across the globe.

a. The nutrients and conditions for bacteria's activity are made possible by adding biochemical media which is used for the tests.

b. The consultant inoculates the test media with bacteria using thoughtful techniques to keep the samples clean and uncontaminated.

c. Media in which bacteria were added are placed at the right temperature and kept for a particular time interval to allow growth and chemical reactions to happen.

d. After incubating, the media should be checked for any differences like color change, gas bubbles or the build-up of precipitate, to know the outcome.

Testing for Antibiotic Resistance in bacteria involves growing them in various concentrations which is done either manually or by an automated process [7]. Every method has its own benefits and drawbacks and accurate testing of organisms is included.

Seeing the growth of organisms in culture is a traditional technique and this approach is applied to tests on Drug Susceptibility, Bioassays and work concerning Antibiotics Against Host Defense [8]. Disk-diffusion, Well Diffusion and Broth or Agar Dilution are some of the commonly known and widely used Bioassays.

4. OVERVIEW OF BIOCHEMICAL TESTS

Multiple tests based on Biochemical Reactions are accessible for finding which bacteria are present in the specimen; each test targets a certain enzyme or pathway.

a. CARBOHYDRATE UTILIZATION TESTS

Using pH indicators, color changes and detecting gas production are some ways to find out about Carbohydrate Utilization [9]. Acid made in test tube can be spotted by the change in phenol red and gas that builds up is trapped by Durham Tube during fermentation. Many diagnostic test kits such as API-20 and Enterotube, offer Citrate Utilization Medium as one of their options [10]. The MR-VP Broth that contains 0.5% glucose, is used for the Voges-Proskauer test to check the results from

organisms that make acidic fermentation products [11].

b. METABOLIC BY-PRODUCT TESTS

Indole, a metabolic byproduct from Bacterial Tryptophanase degradation, can be detected by adding Kovac's Reagent to Tryptophan-rich cultures, resulting in a red-colored complex. Hydrogen Sulfide (H_2S) production, a crucial bacterial metabolic indicator, is frequently measured using growth media enriched with iron salts and heavy metal salts.

5. ENZYME ACTIVITY TESTS

Enzyme activity tests are critical for identifying bacteria based on their enzymatic capabilities, providing valuable insights into their metabolic pathways and virulence factors. Tests for bacterial enzymes are shown in the table below:

Table 1: Summary of Key Biochemical Tests in Bacterial Identification

Test	Principle	Methodology	Applications
Catalase	Catalase decomposes H_2O_2 into H_2O and O_2	Mix a colony with H_2O_2; rapid O_2 bubble formation = positive [12]	Differentiates Catalase-Positive (e.g., *Staphylococcus*) vs. Negative (e.g., *Streptococcus*) [12, 13, 14]
Oxidase	Cytochrome C Oxidase oxidizes a chromogenic reducer (e.g., TMPD), yielding a colored product	Rub colony on filter/swab impregnated with TMPD; blue/purple within seconds = positive [15, 16]	Identifies Oxidase-Positive Gram-Negatives such as *Pseudomonas* and *Neisseria*
Coagulase	Coagulase activates Prothrombin → Fibrin clot formation	Slide test for bound Coagulase/clumping factor; tube test for Free Coagulase	Key test for *Staphylococcus aureus* [17]
Urease	Urease hydrolyzes urea → NH_3 + CO_2, raising pH	Inoculate into urea medium with Phenol Red; pink color (alkaline shift) = positive [18]	Detects urease-producers like *Proteus*, *Klebsiella*, *Helicobacter pylori* [11]
Indole	Tryptophanase breaks down tryptophan → indole, pyruvate, NH_3	Grow in tryptophan-rich broth; add Kovac's Reagent; red ring = indole production	Differentiates *E. coli* (indole-positive) from other Enterobacteriaceae
Methyl Red (MR)	Detects stable mixed-acid fermentation of glucose → acidic end-products	After growth in glucose broth, add Methyl Red; red = pH < 4.4 (acidic), yellow = pH > 6.2	Distinguishes mixed-acid fermenters in Enterobacteriaceae
Voges-Proskauer (VP)	Detects Acetoin (neutral fermentation product) [19]	After incubation in glucose broth, add α-naphthol + KOH; red color = Acetoin present	Identifies Butanediol fermenters (e.g., *Enterobacter*, *Serratia*) in Enterobacteriaceae [20]
Citrate Utilization	Citrate Permease allows use of citrate as sole C-source, producing alkaline byproducts	Inoculate Simmons Citrate agar (bromothymol blue); blue = citrate utilization [21]	Differentiates Citrate-Positive (e.g., *Klebsiella*, *Citrobacter*) vs. Citrate-Negative (e.g., *E. coli*) [22]
TSI Agar	TSI agar Fermentation of glucose, lactose and sucrose results in H_2S production; changes the pH and causes black precipitate to form.	Stab and streak on TSI slant; yellow butt/slant = acid from sugar fermentation, black = H_2S production	Preliminary ID of enteric Gram-Negatives by sugar fermentation pattern and H_2S production
H_2S Production	Enzymatic reduction of sulfur compounds → H_2S gas reacts with iron salts → black precipitate [23, 24]	Inoculate on SIM, KIA, or Sulfide Indole Motility media containing sodium thiosulfate & ferrous sulfate; blackening = H_2S [25, 26]	Differentiates H_2S-producers (e.g., *Salmonella*, *Proteus*) among enterics

6. SPECIALIZED BIOCHEMICAL TESTS

Specialized assays are used to enhance bacterial identification and characterization, focusing on unique enzymatic activities, metabolic pathways, or structural components specific to specific bacterial groups.

a. **CAMP Test (Christie–Atkins–Munch-Peterson):** The CAMP test, named after Christie, Atkins, and Munch-Petersen, is primarily used to identify *Streptococcus agalactiae* based on its ability to produce the CAMP factor, a diffusible, heat-stable protein that acts synergistically with the beta-lysin of *Staphylococcus aureus* to cause an amplified zone of hemolysis on blood agar, a characteristic reaction facilitating the presumptive identification of *Streptococcus agalactiae*.

b. **Bile Esculin Test:** The presence of Esculinase in bacteria is revealed in these tests when they digest Esculin, forming Esculetin and glucose and the treatment with ferric ions turns the mixture dark brown or black, proving a positive reaction [27].

c. **Niacin Test:** Also known as the Niacinamide Test, is predominantly employed to differentiate *Mycobacterium tuberculosis* from other mycobacteria, relying on the detection of free Niacin (Nicotinic acid) production, a metabolic byproduct characteristically produced by *M. tuberculosis*, which, upon reaction with cyanogen bromide and an aromatic amine, yields a yellow-colored complex indicative of a positive result [28].

7. AUTOMATION AND ADVANCES IN BIOCHEMICAL TESTING

Automated biochemical testing platforms have significantly improved efficiency, reproducibility, and throughput in clinical diagnostics and microbiological research. These systems integrate multiple biochemical tests into a single unit, allowing simultaneous evaluation of multiple bacterial characteristics with minimal manual intervention [29]. Automation reduces human error and accelerates turnaround times; enabling clinicians to make informed patient care decisions. Because of its high accuracy, time efficiency and cost, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has taken the place of traditional biochemical tests in bacterial identification [30].

7.1. MODERN BIOCHEMICAL IDENTIFICATION TECHNIQUES:

7.1.1. MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry):

MALDI-TOF mass spectrometry greatly improves the process of identifying bacteria and fungi in the clinic [31]. It automates how jobs are done and recognizes bacterial species faster by analyzing the proteins present in the microbes [32]. Quick incubation periods on solid media and MALDI-TOF MS have been found to be useful for identifying pathogens found in positive blood cultures [33].

7.1.2. Molecular Diagnostics:

Bacterial identification is made much more sensitive and efficient when scientists use molecular diagnosis, as it beats traditional approaches in terms of accuracy, detail and time. They help find and describe pathogens quickly which allows patients to be treated more effectively [34]. Tests that amplify nucleic acids such as Polymerase Chain Reaction, help in quick and accurate identification of bacterial pathogens . Multiplex PCR (Polymerase Chain Reaction) assays make it possible to find several pathogens or genes at once from a single specimen which speeds up testing and supports proper treatment [35].

7.1.3. Fatty Acid Profiling:

Thanks to Gas Chromatography-Fatty Acid Methyl Ester analysis, it is possible to identify and group bacterial species by looking at their fatty acids which gives insights into their genetics and physiology [36].

7.1.4. NGS (Next-Generation Sequencing):

Metagenomic next-generation sequencing represents a significant advancement in identifying infectious diseases by amplifying and sequencing all nucleic acids in a sample, enabling microbial identification without needing prior knowledge of potential pathogens [37]. Next-generation sequencing can sequence small genomes, such as bacterial genomes, in a few days. Whole-genome sequencing offers unambiguous data pertaining to complete analysis of the entire genome and may ultimately become a highly powerful tool in routine clinical settings [38]. Several PCR arrays are available to detect the most frequent pathogens associated with bloodstream infections and their major Antimicrobial Resistance Genes [39]. The integration of molecular techniques into clinical microbiology laboratories has greatly improved the speed and accuracy of pathogen identification, leading to improved patient outcomes and enhanced infection control measures [40].

8. QUALITY CONTROL AND STANDARDIZATION

Using Quality Control and following set protocols ensures that the results of Biochemical Tests used to find bacteria in clinical and research spaces are always dependable, accurate and consistent. Controls help determine that a test has been done properly and can find mistakes that may have occurred during the procedure [41]. It is very important to keep laboratory equipment such as Spectrophotometers, Incubators, Autoclaves and Automated Systems in good condition by undertaking regular maintenance and calibration.

9. LIMITATIONS OF BIOCHEMICAL TESTS

Though essentially important in microbiology, phenotypic approaches to identifying microorganisms can be less precise and less useful in modern medical and research settings, especially when similar or unfamiliar species are involved. Some key limitations are shown in Table 2.

Table 2: Shortcomings of Traditional Biochemical Tests in Modern Microbial Diagnostics

Limitation	Explanation
1. Consume more time [42]	Most biochemical tests take 24–72 hours to complete, so it delays the process of diagnosis.
2. Less sensitivity for few organisms [43]	Cannot detect low bacterial loads or VBNC (Viable But Non-Culturable) organisms.
3. Phenotypic overlap [42]	Two animals similar in biology could have similar biochemical features, so they are mistaken for one another.
4. Environmental influence [43]	Things such as pH, temperature, and the components of the media control enzymes and can change the quality of the results.
5. Not suitable for fastidious/anaerobic organisms [44]	A lot of pathogens cannot grow in the normal biochemical media that are used.
6. Labor-intensive and subjective [45]	Visual interpretation (color changes, precipitates) can lead to inter-observer variability.
7. Limited detection of resistance genes or virulence factors [46]	Cannot tell if the bacteria can harm people by being immune to antibiotics or producing dangerous chemicals.

Table 3: Barriers to Molecular Diagnostics in Resource-Limited Environments

Challenge	Explanation
1. High initial cost [47]	At times, essential equipment such as PCR and sequencers is very expensive and hard to use in places with limited resources.
2. Need for skilled personnel [48]	Using molecular assays often needs skilled workers in the laboratory, which are often missing in places with low incomes.
3. Infrastructure limitations [44]	Since there are problems with electricity, refrigerators, and water, it is hard to rely on molecular tests.
4. Reagent and supply chain dependency [45]	It is not always possible to find or pay for essential reagents and consumables in all cases.
5. Risk of contamination [46]	If procedures are not carried out carefully, tests often generate false positives in patients with high sensitivity.
6. Lack of regulatory oversight [49]	In particular areas, there are not national rules for checking the quality of molecular tests.

Table 4: Need for Complementary Use of Traditional Tests Alongside Molecular Diagnostics

Reason	Explanation
1. Cost-effective preliminary identification [43]	If molecular tools cannot be used, biochemical test results are quite affordable.
2. Robust and adaptable to low-resource settings [45]	Just a basic setup is needed; this is good during emergencies or in small clinics.
3. Recommended by WHO as part of tiered diagnostic strategy [49]	According to WHO, the use of various tools and tests should depend on what is available in each place.
4. Essential for culture-based antibiotic susceptibility testing (AST) [42]	This technique, AST, still depends on samples that are grown by biochemical or culture means.
5. Used in combination with POC diagnostics [46]	Sometimes, POC tests include basic biochemical changes, like changes in color because of an enzyme reaction.

12. CLINICAL SIGNIFICANCE AND APPLICATIONS

Accurate bacterial identification is crucial in clinical microbiology laboratories for effective diagnosis, treatment, and management of infectious diseases, improving patient outcomes and informing clinical decision-making [50]. Rapid identification is especially important in bloodstream infections.

13. FUTURE DIRECTIONS AND CONCLUDING REMARKS

Bacterial identification is undergoing a transformation due to innovative technologies and recognition of limitations in traditional methods. Advanced molecular techniques like whole-genome sequencing and metagenomics are revolutionizing identification, providing resolution and insights into antimicrobial resistance mechanisms. This transformation is especially impactful considering that antibiotic prescriptions are too often not based on definitive diagnosis, which can lead to patients receiving inappropriate treatment. The evolution of clinical microbiology is driven by technological advancements, from the initial use of light microscopes to modern data-intensive methods. New laboratory techniques necessitate updating sample processing steps. AI, automation, and digital platforms will optimize operations, improve diagnostic precision, and enhance patient outcomes, contributing to pandemic preparedness. The importance of laboratory practices in a patient-oriented approach is increasingly recognized, optimizing technology advances for improved patient care.

REFERENCES:

[1]. Li H, Torab P, Mach KE, Surrette C, England MR, Craft DW, et al. Adaptable microfluidic system for single-cell pathogen classification and antimicrobial susceptibility testing. Proc Natl Acad Sci U S A. 2019;116(21):10270–9.

[2]. Faron ML, Buchan BW, Hyke J, Madisen N, Lillie JL, Granato PA, et al. Multicenter

evaluation of the Bruker MALDI Biotyper CA system for identification of clinical aerobic gram-negative bacterial isolates. PLoS One. 2015;10(11):e0141350.

[3]. Bhattacharyya RP, Walker M, Boykin R, Son SS, Liu J, Hachey AC, et al. Rapid identification and phylogenetic classification of diverse bacterial pathogens in a multiplexed hybridization assay targeting ribosomal RNA. Sci Rep. 2019;9(1):4516.

[4]. Endimiani A, Ramette A, Rhoads DD, Jacobs MR. The evolving role of the clinical microbiology laboratory in identifying resistance in Gram-negative bacteria: an update. Infect Dis Clin North Am. 2020;34(4):659–76.

[5]. Semret M, Ndao M, Jacobs J, Yansouni CP. Point-of-care and point-of-'can': leveraging reference-laboratory capacity for integrated diagnosis of fever syndromes in the tropics. Clin Microbiol Infect. 2018;24(8):836–44.

[6]. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect. 2015; 6:22–9.

[7]. Sanchini A. Recent developments in phenotypic and molecular diagnostic methods for antimicrobial resistance detection in Staphylococcus aureus: a narrative review. Diagnostics (Basel). 2022;12(1):208.

[8]. Franco-Duarte R, Černáková L, Kadam S, Kaushik KS, Salehi B, Bevilacqua A, et al. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019;7(5):130.

[9]. Elston HR, Baudo JA, Stanek JP, Schaab M. Multi-biochemical test system for distinguishing enteric and other gram-negative bacilli. Appl Microbiol. 1971;22(3):408–14.

[10]. MacWilliams MP. Citrate test protocol. Am Soc Microbiol. 2009:1–7.

[11]. Rahman MA, Ahmad T, Mahmud S, Barman NC, Haque MS, Uddin ME, et al. Isolation, identification and antibiotic sensitivity pattern of Salmonella spp. from locally isolated egg samples. Am J Pure Appl Sci. 2019;1(1):1–1.

[12]. Taylor WI, Achanzar D. Catalase test as an aid to the identification of Enterobacteriaceae. Appl Microbiol. 1972;24(1):58–61.

[13]. Heston TF. A case study in blockchain health care innovation. Int J Curr Res. 2017;9(11):60587–8.

[14]. Al-Kharousi ZS, Al-Ramadhani Z, Al-Malki FA, Al-Habsi N. Date vinegar: first isolation of Acetobacter and formulation of a starter culture. Foods. 2024;13(9):1389.

[15]. Barer MR, Marsh PJ. Rapid cytochemical demonstration of cytochrome oxidase activity in pathogenic bacteria. J Clin Pathol. 1992;45(6):487–9.

[16]. Jurtschuk JR, McQuitty DN. Survey of oxidase-positive and-negative bacteria using a quantitative Kovacs oxidase test. Int J Syst Evol Microbiol. 1976;26(2):127–37.

[17]. Giuliano C, Patel CR, Kale-Pradhan PB. A guide to bacterial culture identification and results interpretation. P T. 2019;44(4):192.

[18]. Isenberg HD. Clinical Microbiology Procedures Handbook. Washington DC: American Society for Microbiology; 1992.

[19]. Rasouli Z, Abdollahi H, Maeder M. Generalized indicator-based determination of solution pH. Anal Chim Acta. 2020; 1109:90–7.

[20]. Cox NA, Thomson JE, Bailey JS. Minitek inoculum broth for testing indole production by Enterobacteriaceae. J Food Prot. 1981;44(6):445–6.

[21]. Di C, Yu W, Lu Y. Screening of methanotrophic strain for scale applications: methane emission reduction and resource utilization. Sustainability. 2025;17(8):3687.

[22]. Blount ZD, Maddamsetti R, Grant NA, Ahmed ST, Jagdish T, Baxter JA, et al. Genomic and phenotypic evolution of Escherichia coli in a novel citrate-only resource environment. Elife. 2020;9: e55414.

[23]. Padron AP, Dockstader WB. Selective medium for hydrogen sulfide production by salmonellae. Appl Microbiol. 1972;23(6):1107–12.

[24]. Küster E, Williams ST. Production of hydrogen sulfide by streptomycetes and methods for its detection. Appl Microbiol. 1964;12(1):46–52.

[25]. McDonough PL, Shin SJ, Lein DH. Diagnostic and public health dilemma of lactose-fermenting Salmonella enterica serotype Typhimurium in cattle in the Northeastern United States. J Clin Microbiol. 2000;38(3):1221–6.

[26]. SN. Isolation of Salmonella enterica serovar. 2014.

[27]. Franklin ML, Clark WA. Simple, inexpensive, and rapid way to produce Bacillus subtilis spores for the Guthrie bioassay. J Clin Microbiol. 1981;14(1):113–5.

[28]. Matsuo H, Hanamure Y, Miyano R, Takahashi Y, Ōmura S, Nakashima T. Screening for sulfur compounds by molybdenum-catalyzed oxidation combined with liquid chromatography-mass spectrometry. Molecules. 2020;25(2):240.

[29]. Starr SE, Thompson FS, Dowell VR Jr, Balows A. Micromethod system for identification of anaerobic bacteria. Appl Microbiol. 1973;25(5):713–7.

[30]. Zengin Canalp H, Bayraktar B. Direct rapid identification from positive blood cultures by MALDI-TOF MS: specific focus on turnaround times. Microbiol Spectr. 2021;9(3): e01103-21.

[31]. Ping Y, Chen Q, Sun X, Wang H, Lin S, Zhu B, et al. Clinical evaluation of an in-house Xpert lysate-based method combined with MALDI-TOF MS for the rapid identification of positive blood cultures. BMC Microbiol. 2025;25(1):1–2.

[32]. Clark CM, Murphy BT, Sanchez LM. A call to action: the need for standardization in developing open-source mass spectrometry-based methods for microbial subspecies discrimination. mSystems. 2020;5(1):e00128-19.

[33]. Kohlmann R, Hoffmann A, Geis G, Gatermann S. MALDI-TOF mass spectrometry following short incubation on a solid medium is a valuable tool for rapid pathogen identification from positive blood cultures. Int J Med Microbiol. 2015;305(4–5):469–79.

[34]. Procop GW. Molecular diagnostics for the detection and characterization of microbial pathogens. Clin Infect Dis. 2007;45(Suppl 2):S99–111.

[35]. Senok A, Dabal LA, Alfaresi M, Habous M, Celiloglu H, Bashiri S, et al. Clinical impact of the BIOFIRE blood culture identification 2 panel in adult patients with bloodstream infection: a multicentre observational study in the United Arab Emirates. Diagnostics (Basel). 2023;13(14):2433.

[36]. Maeda Y, Sugiyama Y, Kogiso A, Lim TK, Harada M, Yoshino T, et al. Colony fingerprint-based discrimination of Staphylococcus species with machine learning approaches. Sensors (Basel). 2018;18(9):2789.

[37]. Xu F, Chen C, Lu S, Xue M, Ding H, Song Y, et al. Impact of metagenomics next-generation sequencing on etiological diagnosis and early outcomes in sepsis. J Transl Med. 2025;23(1):394.

[38]. Al-Zahrani IA. Routine detection of carbapenem-resistant gram-negative bacilli in clinical laboratories: a review of current challenges. Saudi Med J. 2018;39(9):861–8.

[39]. Jacobs MR, Colson JD, Rhoads DD. Recent advances in rapid antimicrobial susceptibility testing systems. Expert Rev Mol Diagn. 2021;21(6):563–78.

[40]. Piddock LJ. Assess drug-resistance phenotypes, not just genotypes. Nat Microbiol. 2016;1(8):16028.

[41]. Ceuppens S, Delbeke S, De Coninck D, Boussemaere J, Boon N, Uyttendaele M. Characterization of the bacterial community naturally present on commercially grown basil leaves: evaluation of sample preparation prior to culture-independent techniques. Int J Environ Res Public Health. 2015;12(8):10171–97.

[42]. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. 12th ed. St. Louis: Mosby Elsevier; 2007. p. 778–81.

[43]. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2, 2nd ed. Cambridge: Cambridge University Press; 2006. p. 167.
[44]. Ombelet S, Ronat JB, Walsh T, Yansouni CP, Cox J, Vlieghe E, et al. Clinical bacteriology in low-resource settings: today's solutions. Lancet Infect Dis. 2018;18(8):e248–58.
[45]. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect. 2010;16(8):1062–9.
[46]. Yager P, Domingo GJ, Gerdes J. Point-of-care diagnostics for global health. Annu Rev Biomed Eng. 2008; 10:107–44.
[47]. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010;363(11):1005–15.
[48]. Niemz A, Ferguson TM, Boyle DS. Point-of-care nucleic acid testing for infectious diseases. Trends Biotechnol. 2011;29(5):240–50.
[49]. World Health Organization. Global Diagnostics Landscape and Priorities. Geneva: WHO; 2021.
[50]. Damor RR, Kubavat AR. Antibiotic sensitivity profile of Klebsiella isolates and its impact on clinical outcome.

Cite of article: Karmakar S, Rehan A, Jahan R, Mahanoor. Biochemical and molecular approaches to bacterial pathogen identification: traditional techniques and emerging advances. Int J Med Lab Res. 2025;10(2):34–42.
http://doi.org/10.35503/IJMLR.2025.10205

CONFLICT OF INTEREST: Authors declared no conflict of interest
SOURCE OF FINANCIAL SUPPORT: Nil