MODERN METHODS OF DIAGNOSING BACTERIAL INFECTIONS

Begimkulova Nigina
Cadet of the department of clinical laboratory diagnosis with the course of clinical laboratory diagnostics of PGD;
Nurmatova M.A.
Assistant of the department of clinical laboratory diagnosis with the course of clinical laboratory diagnostics of PGD;
Samarkand state medical university
Samarkand, Uzbekistan

Bacterial infections are one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization, more than 700 million cases of bacterial infections are reported annually, with more than 6 million deaths associated with complications from bacterial diseases [1, 2]. Among the most significant pathogens are Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, which cause both hospital-acquired and community-acquired infections.

Modern medicine faces several key challenges in the field of bacterial infections. First, there is the rise of antibiotic resistance, when conventional treatment regimens become ineffective. For example, the spread of methicillin-resistant strains of S. aureus (MRSA) and extended-spectrum beta-lactamase (ESBL) producers significantly complicates treatment and increases the risk of fatal outcomes [3, 4]. Second, early detection of infections is becoming critically important for preventing serious complications such as sepsis, tissue necrosis, and multiple organ failure.

The diagnosis of bacterial infections plays a key role not only in the selection of effective therapy, but also in epidemiological control and prevention of the spread of pathogens. Traditional culture methods, despite their high specificity, have significant limitations: the time it takes to obtain results, dependence on the quality of the material, and the difficulty of identifying rare microorganisms [5].

Modern diagnostic technologies are aimed at reducing the time to diagnosis, increasing the accuracy and sensitivity of methods, and detecting the resistance of microorganisms to antibiotics at an early stage. Such methods include molecular approaches (PCR, LAMP), automated identification and sensitivity testing systems, mass spectrometry, next-generation sequencing, and portable diagnostic devices [6–8].

The aim of this work is to systematize modern approaches to the diagnosis of bacterial infections, describe their principles, advantages, and limitations, and evaluate promising areas of development. Such a systematic review will allow us to present the current state of diagnostics, identify key problems, and demonstrate ways to optimize the process of detecting bacterial pathogens to improve the effectiveness of therapy and prevention of infections.

Classic methods for diagnosing bacterial infections form the basis of clinical microbiology and remain an essential part of laboratory testing for patients. Despite the active introduction of molecular genetic and automated technologies, traditional approaches are highly clinically significant, accessible, and evidence-based. They allow not only to identify the pathogen, but also to assess its morphological, cultural, and biochemical properties [5].

Microscopy is one of the earliest and most accessible methods for diagnosing bacterial infections. The examination of clinical material (blood, sputum, urine, cerebrospinal fluid, smears from the sites of infection) allows preliminary information to be obtained about the presence and nature of the bacterial process. Gram staining, based on differences in the structure of the bacterial cell wall, is the most widely used method [1].

Gram-positive microorganisms are characterized by a thick peptidoglycan shell and stain purple, while Gram-negative bacteria, which have an outer membrane, stain pink. This approach allows the doctor to tentatively determine the group of the pathogen and begin empirical antibiotic therapy [2]. In addition to Gram staining, special staining methods are used: Ziehl-Neelsen staining - to detect acid-fast bacteria (e.g., Mycobacterium tuberculosis); Romanowsky-Giemsa staining - for suspected intracellular bacteria; fluorescence microscopy - to increase the sensitivity of the method [6].

However, microscopy has a number of limitations: low sensitivity at low bacterial concentrations, inability to accurately identify species, and dependence of the result on the quality of the sample and the experience of the specialist [7].

Cultural methods remain the “gold standard” for laboratory diagnosis of bacterial infections. They are based on the cultivation of microorganisms on nutrient media, followed by identification and determination of antibiotic sensitivity [3].

Depending on the suspected pathogen, different types of nutrient media are used: universal (meat peptone agar, blood agar); selective and differential diagnostic media (Endo, MacConkey); enriched media for fastidious microorganisms [8]. Culture testing allows for the evaluation of: colony morphology; growth characteristics; hemolytic properties; biochemical activity of bacteria.

The main advantage of this method is the ability to perform antibiotic susceptibility testing, which is especially important in the context of growing antibiotic resistance [9]. However, a significant disadvantage of culture-based methods is the length of the test, which can take from 24 hours to several days, and for slow-growing microorganisms, up to several weeks [4].

After isolation of a pure culture, biochemical identification is performed based on the ability of microorganisms to ferment various substrates and produce specific enzymes. Classic biochemical tests include the determination of: catalase; oxidase; urease; ability to ferment carbohydrates; production of hydrogen sulfide and indole [10].

Modern laboratories use standardized biochemical panels that reduce identification time and increase the reproducibility of results. However, biochemical methods require a viable culture and may be less accurate with atypical strains [11].

Serological diagnosis is based on the detection of specific antibodies or antigens in the patient's blood serum. These methods are particularly relevant in cases where direct isolation of the pathogen is difficult or impossible [12]. The most common serological reactions are: agglutination reaction; complement fixation reaction; enzyme-linked immunosorbent assay (ELISA); latex agglutination [5].

Serological methods allow for retrospective diagnosis and epidemiological studies. However, their limitations include the late appearance of antibodies, the possibility of cross-reactions, and difficulties in interpreting results in patients with immunodeficiencies [13].

Despite the emergence of high-tech methods, classical approaches remain indispensable. They are widely used in clinical practice due to their relative accessibility, low cost, and high specificity. In most cases, classical methods are used as the first stage of diagnosis, supplementing and confirming the data of molecular and automated studies [14].

In modern clinical practice, methods for rapid and automated diagnosis of bacterial infections are of particular importance. The need for early initiation of etiotropic therapy, especially in severe conditions (sepsis, meningitis, hospital infections), requires a reduction in the time from biomaterial collection to obtaining reliable results. Unlike classical methods, these technologies are aimed at accelerating the identification of the pathogen and increasing the accuracy of laboratory tests [15].

Rapid diagnostics is a group of methods that allow results to be obtained within a few minutes or hours. These methods include immunochromatographic tests, latex agglutination, and rapid serological reactions. Their principle is based on the detection of specific antigens of bacterial pathogens or antibodies in clinical material [5].

Rapid tests are widely used in the diagnosis of bacterial respiratory tract infections, intestinal infections, meningitis, and sexually transmitted infections. The main advantages of these methods are: ease of use; minimal laboratory equipment requirements; applicability in emergency care settings; and rapid preliminary results [16].

However, rapid methods have limited sensitivity and specificity compared to culture and molecular methods. They do not allow the antibiotic sensitivity of the pathogen to be determined and often require confirmation by other laboratory tests

[17].

The automation of microbiological diagnostics has become an important stage in the development of laboratory medicine. Modern automated systems allow for the standardization of processes for culturing, identifying microorganisms, and determining their sensitivity to antibacterial drugs [6].

The principle of operation of such systems is based on the analysis of the biochemical activity of bacteria, changes in the optical characteristics of the medium, or the recording of metabolic processes. The use of automated analyzers significantly reduces the influence of the human factor and increases the reproducibility of results [18]. The advantages of automated systems include: reduced pathogen identification time; high accuracy and standardization of analysis; the ability to simultaneously examine a large number of samples; automatic generation of antibioticograms [9].

Despite their obvious advantages, these systems require significant financial investment and regular maintenance, which limits their use in laboratories with limited resources [15].

Over the past few decades, molecular diagnostic methods have become one of the key tools in laboratory practice due to their high sensitivity, specificity, and speed of results. Unlike classical and automated methods, molecular technologies are based on the detection of pathogen nucleic acids, which allows the diagnosis of infectious processes even with a minimal amount of microorganisms or when it is impossible to culture them [7].

The particular value of molecular diagnostics lies in the ability to detect bacterial infections early, determine the etiological agent, and identify virulence and antibiotic resistance genes, which is of fundamental importance for personalized therapy [12].

Polymerase chain reaction is one of the most widely used molecular methods in clinical microbiology. The method is based on the repeated amplification of a specific fragment of bacterial DNA using primers, DNA polymerase, and cyclic temperature changes [8].

PCR allows bacterial DNA to be detected directly in clinical material (blood, cerebrospinal fluid, urine, smears), bypassing the cultivation stage. This significantly reduces the time required for diagnosis — from several hours to one working day [7]. The advantages of PCR include high sensitivity and specificity; the possibility of early diagnosis; detection of difficult-to-culture and intracellular bacteria; and versatility of application [9].

Next-generation sequencing (NGS) is a revolutionary approach to the diagnosis of infectious diseases. The method allows the complete nucleotide sequence of a bacterial genome to be determined or the total genetic material (metagenomics) to be analyzed directly from a clinical sample [12]. NGS is used for: identifying rare and uncultivable bacteria; analyzing antibiotic resistance genes; studying outbreaks of infections; molecular epidemiological monitoring [17].

Metagenomic analysis is especially valuable in cases where standard diagnostic methods do not yield results, for example, in infections of unknown etiology or in patients with severe immunodeficiencies [18]. The limitations of NGS remain its high cost, the complexity of data interpretation, and the need for a developed bioinformatics infrastructure [19].

Biosensors are innovative analytical devices that combine a biological recognition element (enzymes, antibodies, DNA probes) and a physical signal transducer. They allow the detection of bacterial cells, toxins, or nucleic acids in real time [20].

Modern biosensors are highly sensitive and can be used for rapid diagnosis in clinical, sanitary-epidemiological, and field conditions. Their main advantage is the ability to obtain results without complex laboratory infrastructure [21].

Microfluidic technologies ("lab-on-a-chip") allow a complete analysis cycle, from sample preparation to detection, to be performed on a single miniature device. Such systems require a minimal amount of biomaterial and reagents, making them particularly promising for rapid diagnostics [22]. Microfluidic platforms are integrated with molecular and immunochemical methods, ensuring high speed and accuracy of analysis. Their use is considered one of the key areas of development in personalized medicine [23].

Current trends are focused on the integration of molecular and phenotypic methods, the development of rapid tests to detect resistance directly at the patient's bedside, and the introduction of digital data analysis systems [24].

The development of personalized medicine involves the individual selection of antibiotic therapy based on a comprehensive assessment of the pathogen's genome and the patient's clinical characteristics, making the diagnosis of antibiotic resistance one of the key areas of modern microbiology [23].

Modern diagnosis of bacterial infections is a combination of classical methods and new technologies aimed at increasing the speed, accuracy, and individualization of therapy. The transition from traditional culture-based approaches to molecular and high-throughput methods allows for improved clinical outcomes, especially in the context of growing antibiotic resistance.

References:

1. Abduhakimov B. A. et al. Bolalar va o'smirlarda birlamchi tuberkulyozning o'ziga xos kechish xususiyatlari va klinik-laboratoriya usullari //Ta'lim innovatsiyasi va integratsiyasi. – 2024. – T. 32. – №. 3. – C. 139-143.
2. Бердиярова Ш. Ш. и др. Клинико-лабораторная диагностика фолиевой кислотодефицитной анемии //TADQIQOTLAR. UZ. – 2024. – T. 49. – №. 3. – C. 46-53.

3. Umarova T. A., Kudratova Z. E., Axmadova P. Role of conditionally pathogenic microflora in human life activities //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – Т. 2. – №. 11. – С. 29-32.
4. Muhamadiyeva L. A., Kudratova Z. E., Sirojeddinova S. Pastki nafas yo'llari patologiyasining rivojlanishida atipik mikrofloraning roli va zamonaviy diagnostikasi //Tadqiqotlar. Uz. – 2024. – Т. 37. – №. 3. – С. 135-139.
5. Umarova T. A., Kudratova Z. E., Norboyeva F. Modern aspects of etiology and epidemiology of giardias //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – Т. 2. – №. 11. – С. 25-28.
6. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 117-120.
7. Umarova T. A., Kudratova Z. E., Maxmudova H. Mechanisms of infection by echinocococosis //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – Т. 2. – №. 11. – С. 18-21.
8. Даминов Ф. А., Исомадинова Л. К., Рашидов А. Этиопатогенгетические и клинико-лабораторные особенности сальмонелиоза //TADQIQOTLAR. UZ. – 2024. – Т. 49. – №. 3. – С. 61-67.
9. Umarova T. A., Kudratova Z. E., Baxromova M. Autoimmune diseases: new solutions in modern laboratory diagnostics //International Conference on Modern Science and Scientific Studies. – 2024. – С. 78-81.
10. Бердиярова Ш. Ш. и др. Узловой зоб и его клинико-лабораторная диагностика //TADQIQOTLAR. UZ. – 2024. – Т. 49. – №. 3. – С. 38-45.
11. Umarova T. A., Kudratova Z. E., Muhsinovna R. M. The main purpose of laboratory diagnosis in rheumatic diseases //International Conference on Modern Science and Scientific Studies. – 2024. – С. 82-85.
12. Umarova T. A., Kudratova Z. E., Ruxshona X. Contemporary concepts of chronic pancryatitis //International Conference on Modern Science and Scientific Studies. – 2024. – С. 11-15.
13. Хамидов З. З., Амонова Г. У., Исаев Х. Ж. Некоторые аспекты патоморфологии неспецифических язвенных колитов //Молодежь и медицинская наука в XXI веке. – 2019. – С. 76-76.
14. Umarova T. A., Kudratova Z. E., Muminova G. Instrumental diagnostic studies in chronic pancreatitis //International Conference on Modern Science and Scientific Studies. – 2024. – С. 16-20.
15. Атамурадовна М.Л., Рустамовна Р.Г., Эркиновна К.З. Роль современных биомаркеров в изучении различных поражений головного мозга //Достижения науки и образования. – 2020. – №. 10 (64). – С. 88-90.

16. Рустамова Г. Р., Мухамадиева Л. А. Современные аспекты клинико-лабораторных методов исследования острой ревматической лихорадки //International scientific review. – 2020. – №. LXVI. – С. 106-110.
17. Кудратова З.Е. и др. Роль цитокиновой регуляции при обструктивном синдроме атипичного генеза у детей // Анналы Румынского общества клеточной биологии. – 2021. – Т. 25. – №. 1. – С. 6279-6291.
18. Erkinovna K. Z. et al. Bronchial obstruction syndrome in young children with respiratory infections of different etiology: features of clinical manifestations and immune response //Проблемы науки. – 2021. – №. 1 (60). – С. 60-62.
19. Кудратова З.Е. и др. Хламидийные инфекции (внутриклеточная инфекция) в развитии бронхита // TJE-Tematics journal of Education ISSN. – 2021. – С. 2249-9822.
20. Kudratova Z. E. et al. Principles of therapy of chlamydial and mycoplasma infections at the present stage //Вопросы науки и образования. – 2021. – №. 28 (153). – С. 23-26.
21. Rustamova G. R., Kudratova Z. E. CHRONIC ENDOMETRITIS OLD ISSUES NEW POSSIBILITIES //Western European Journal of Medicine and Medical Science. – 2024. – Т. 2. – №. 5. – С. 12-14.
22. Erkinovna K. Z., Rustamovna R. G., Suratovna H. F. LABORATORY MARKERS OF PERINATAL HYPOXIC DAMAGE TO THE CENTRAL NERVOUS SYSTEM IN NEWBORNS //Наука, техника и образование. – 2020. – №. 10 (74). – С. 102-104.
23. Mukhamadieva L. A., Rustamova G. R., Kudratova Z. E. IMMEDIATE RESULTS OF COMPLEX TREATMENT OF CHILDREN WITH CHRONIC TONSILLITIS AND CHRONIC ADENOIDITIS ASSOCIATED WITH CMV AND EBV //Western European Journal of Medicine and Medical Science. – 2024. – Т. 2. – №. 5. – С. 20-24.
24. Umarova T. A., Kudratova Z. E., Norxujayeva A. Etiopathogenesis and modern laboratory diagnosis of prostatitis //International Conference on Modern Science and Scientific Studies. – 2024. – С. 6-10.