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The Effectiveness of Modern Detection Methods in the Diagnosis of Pathogenic Intestinal Infections and the Prospects for their Improvement

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Abstract: Infectious diseases of the gastrointestinal tract are the main cause of morbidity and mortality in children in developing countries. A wide range of bacteria, viruses, protozoa and parasites can cause diarrhea and other intestinal infections. These infections are usually diagnosed by inoculation, microscopy, and immunoassay with antigen detection. Cultivation and microscopy are procedures that are insufficiently sensitive, time—consuming and require special laboratory equipment and well-trained personnel. However, newer methods of rapid antigen detection and molecular methods are constantly replacing traditional diagnostic methods due to advances in molecular diagnostics and the advent of tests that can be purchased. This review summarizes and discusses the availability, advantages and disadvantages of molecular methods for the detection and identification of human gastrointestinal pathogens. We will look at the advantages and disadvantages of direct and indirect methods of detecting parasites here. Many tests give false positive or false negative results. The tests available for use vary in sensitivity and specificity. Thus, tests for the presence of the pathogen should be carried out, especially in doubtful cases, using all available methods. The methods used should make it possible to distinguish an active infection from one suffered in the past. Finally, we will look at laboratory "case reports" in which we will discuss diagnostic methods that can successfully detect parasites. We will also talk about the possibilities of using artificial intelligence to improve the diagnosis of parasitic diseases.

Keywords: Parasites in the stomach and intestines, pathogens, bacteria, fungi, viruses, food, culture analysis, PCR, immunoassays.

Introduction. Second only to respiratory tract infections, gastrointestinal infections are among the most common infections worldwide. Various bacterial, viral and parasitic pathogens can cause gastrointestinal infections. Non-observance of hygiene rules and consumption of contaminated food or water are the main ways of spreading these infections. Through direct contact or through household items, infections can also be transmitted from person to person. The most common symptom is diarrhea, which usually goes away on its own and in most people who are in good physical shape, it usually goes away within a few days. However, diarrhea can progress in very young children and if hygiene rules are not followed, leading to severe dehydration, malnutrition, bacteremia and other complications that can lead to death. A World Health Organization (WHO) report claims that more than 1.7 billion cases of diarrhea are reported worldwide each year [1, 2, 3,4, 5]. Gastrointestinal parasites (GCP) are among the most common diseases worldwide. However, the fact that most parasitic diseases do not show characteristic symptoms complicates their clinical diagnosis; indeed, in order to make a correct diagnosis, it is necessary to confirm both the presence of the parasite and its connection with the symptoms of the disease. During the diagnosis process, it is also necessary to take into account the possibility that infections may be caused by several coexisting parasites. Thus, when conducting any diagnosis, it is necessary to take into account both the local epidemiological situation and the prevalence of the parasite in a particular population. In some cases, the presence of a parasite may not be associated with the symptoms of the disease. Thanks to recent advances in molecular diagnostics with the possibility of multiplexing, syndromic amplification of nucleic acids can be used to identify many pathogens that cannot be detected by traditional methods [6, 7, 9, 10].

In most countries, most stool testing protocols include a limited list of screening pathogens for acute gastrointestinal infections. Most diagnostic laboratories still use traditional pathogen identification methods, such as enzyme immunoassay for the determination of rotavirus, adenovirus and *Clostridium difficile* toxin, bacterial culture for the determination of shigella and salmonella, as well as direct wet microscopy for a one-year study aimed at studying the epidemiology of common pathogens in the gastrointestinal tract and comparing our results with the causative agents of diarrhea which are reported by our research cooperation centers [11, 12, 13, 14]. The article will consider the advantages and disadvantages of the most common methods of searching for parasites. One group includes direct methods that determine the presence of parasites in the tested material and its species. To achieve this goal, both microscopy and advanced molecular methods can be used, which are aimed at determining the DNA of the parasite. Another category includes indirect methods that may indirectly indicate a parasitic invasion. These methods may be specific (for example, to detect antibodies in blood serum) or non-specific. Different methods should be used in all diagnostic procedures to achieve the most accurate diagnosis, since each test has different sensitivity and specificity and is prone to give false positive or negative results [15,16,17,18].

The main purpose of the presented manuscript is to present a brief review of the literature in the light of the relevance of diagnosis and prospects of modern methods of examination of pathogenic intestinal infection, which is still considered a serious medical problem in connection with the improvement of diagnostic and therapeutic measures.

Cultural, microscopic and immunological traditional methods. For the diagnosis of gastrointestinal infections and enterobacteria, traditional laboratory methods are (1) inoculation and determination of antibiotic sensitivity, (2) microscopic examination of eggs and parasites, and (3) immunoassay to detect antigens [19, 20, 21]. With a number of advantages and disadvantages, traditional sowing remains the gold standard for the diagnosis of bacterial enteropathogens. The main advantage of the seeding method is that it is extremely specific. The specificity of the seeding is one hundred percent if the pathogenic organism is not detected in healthy participants. However, the sensitivity of the seeding varies and is often difficult to determine. The culture method also has the availability of an isolate that can be used to test antibiotic sensitivity. The isolate can be sent to state public health laboratories for further identification, outbreak investigation and epidemiological studies using the traditional cultivation method [22, 23, 24]. To directly detect parasites in the intestine, microscopy of a stool sample can be used to determine the presence of eggs and parasites. However, determining the presence of intestinal parasites by microscopic examination of a direct or stained stool smear is technically difficult and requires highly qualified and experienced personnel. Due to the low concentration of organisms and/or the periodic release of eggs and parasites, their detection may be difficult. The search for antigens using commercially available immunoassays is a popular and simple procedure for detecting certain viruses and intestinal parasites. However, these antigen-based studies are usually not sensitive enough to identify all pathogens that cause gastrointestinal infections [12, 13, 15, 17].

Amplification methods that consist of nucleic acids. In the last ten years, commercially available nucleic acid-based methods have been aimed at identifying either a single pathogen or multiple pathogens using multiplex analysis. Various molecular analyses are available to determine a single pathogen in the stomach. These tests are designed specifically for certain groups of patients. They also comply with medical coding and billing requirements. In addition, these single-component analyses based on nucleic acids allow for certain studies that a doctor may prescribe. Toxic infections of noroviruses and C. difficile are detected using standard single-stage molecular assays [18,19,20]. The most common molecular methods include (1) real-time polymerase chain reaction (PCR), (2) final PCR using massive and microfluidic technologies, and (3) integrated platforms where amplification, extraction, and analysis of nucleic acids are performed in a single step. Isothermal amplification, which does not require thermal cycling, is becoming increasingly popular. These methods of isothermal helicase-dependent amplification are more suitable for the detection of a single pathogen and do not require expensive thermal cycling equipment. [21, 22, 23, 24].

Toxicity testing of *Clostridioides difficile*. Nosocomial and antibiotic-associated diarrhea, as well as pseudomembranous colitis, are caused by *Clostridioides difficile*. Up to 90% of healthy newborns and infants and up to 15% of healthy adults can receive colonization of the gastrointestinal tract of *C. difficile* under normal conditions. Old age, hospitalization, or stay in long-term care facilities are risk factors for developing *C. difficile* disease and usually symptoms of diarrhea appear after antibiotic treatment. The production of binary toxin and large clostridial toxin (TcdA and TcdB) is associated with the pathogenicity of *C. difficile*; strains that do not produce the toxin are considered non-pathogenic. The TcdA gene encodes the toxin A *C. difficile*, which causes diarrhea. Toxin B, encoded by the TcdB gene, destroys cells, causing pseudomembranous colitis. This can lead to complications such as toxic megacolon, colon perforation and sepsis [21,22,23,25]. For laboratory diagnostics of *C. difficile* colonization and disease, stool samples are used to determine the pathogen and its toxins. Initial colonization testing of *C. difficile* is performed using antigen-based immunoassays. For example, toxins A and B can be detected using chromatographic/lateral flow membrane cartridges or enzyme immunoassay (ELISA). In addition, primary screening can be performed using a solid-phase microtiter tablet or a chromatographic/lateral flow membrane cartridge to determine the enzyme glutamate dehydrogenase (GDH) in a patient's stool sample. This method can be used as a separate test, or in combination with a test for toxins A and B. Regardless of the toxigenic nature, the GDH enzyme is produced by all *C. difficile* strains and indicates colonization, but not necessarily an active disease of *C. difficile* [2, 25, 29, 30, 31].

Commercially available multiplex assays for the determination of intestinal pathogens. Many commercial multiplex assays can detect most common pathogens. They can be used both in open systems, where a separate stage of nucleic acid isolation is required, and in closed systems, where nucleic acid isolation, amplification and product analysis occur simultaneously. These studies allow us to identify pathogens that can spread in a certain environment. Before purchasing, the local epidemiological situation and the needs of the institution should be taken into account. Some analyses can identify specific bacterial, viral, and parasitic pathogens. This makes these tests universal for cases where a doctor may request a specific test [25,26,29,30].

BioFire panel for the gastrointestinal tract (GI). The BioFire Gastrointestinal (GI) Panel (BioFire, Salt Lake City, Utah, USA) is a fully integrated system that can detect more bacteria (13 pathogens), viruses (5 pathogens) and parasites (4 pathogens) at the same time than any other analysis. Within one hour, this system simultaneously performs nucleic acid extraction, reverse transcription, amplification and analysis. The technology is based on multiplex PCR amplification, and then the melting curve data is analyzed. The main advantage of BioFire FilmArray is that it can detect most pathogens, as well as the fact that it does not require a lot of time to prepare and process the results. FilmArray has poor performance and an inability to separate bacterial, viral, or parasitic tests if necessary for patients or billing purposes [22,27,31].

The Allplex Panel for Allplex Gastrointestinal Assays (Seegene, Seoul, South Korea) is a new, real-time CE-IVD multiplex PCR assay that detects 13 bacteria, 5 viruses and 6 parasites in four multiplex PCRs. This analysis uses innovative analytical Multiple Temperature Detection (MuDT) technology, which can detect multiple targets in a single fluorescence channel without performing melting curve analysis. The procedure involves separate isolation of nucleic acids from stool samples, and then real-time multiplex PCR using the CFX96TM real-time PCR system (Bio-Rad Laboratories, Richmond, California, USA) for the determination of nucleic acids [29, 30, 31, 32].

Advantages of molecular testing: They offer an improved workflow and faster results with high sensitivity and specificity compared to traditional methods. The multiplexing capability, which allows the identification of multiple intestinal pathogens at the same time, is an additional advantage. Multiplex assays can be especially useful for patients with severe illnesses and in some patient groups that require rapid diagnosis, treatment, and decision-making. From a therapeutic point of view, multiplex molecular assays are useful because they help to avoid unnecessary and inappropriate antimicrobial treatment. This refers to an infection caused by *E. coli* (STEC) producing shiga toxin, in

which antimicrobial treatment may increase the risk of developing hemolytic uremic syndrome (HUS) [11, 15, 18, 19].

Disadvantages of molecular testing: The main obstacles to NAAT are initial setup and costs. However, in the end, replacing traditional cultivation methods with molecular methods often brings benefits. NAAT also cannot distinguish between living and dead organisms, so the results must be carefully interpreted depending on the patient's condition. In addition, a special culture may be required to determine the pathogen and antibiotic sensitivity, depending on the patient, the needs of the doctor and the requirements of the Department of Public Health. An antibiotic sensitivity profile is extremely useful, especially in critical situations where it is necessary to determine whether antibiotic treatment is required and which antibiotic should be used. The transfer of selected bacterial isolates to government public health laboratories is a prerequisite; this plays an important role in public health surveillance, outbreak investigations and monitoring of antibiotic sensitivity [28, 29, 30, 31, 32, 33].

Discussion. In conclusion, traditional diagnostic methods such as microbial culture, hemagglutination inhibition tests and ELISA do not work well with molecular diagnostic technology for the diagnosis of infectious diseases. According to the needs of the user, careful selection and combination of various molecular diagnostic technologies can ensure timely and accurate diagnosis of infectious disease pathogens and facilitate accurate treatment for effective disease control. qPCR technology is mature, affordable and suitable for quantitative and qualitative analysis of common pathogens in standard laboratories. dPCR can be used for absolute quantification of target genes in samples. This method is especially effective for analyzing samples with low pathogen levels, as well as for searching for small mutations and rare target alleles [1, 17, 18, 21, 24]. The gene chip technology allows the simultaneous detection and identification of multiple pathogens. This is especially useful in clinical settings when it comes to mixed infections, when the composition of pathogens is heterogeneous. Unlike gene sequencing technology, which allows the identification of all types and sequences of pathogens, this technology allows the detection of only the genomes of known pathogens and cannot detect new, unknown pathogens. The methods of molecular diagnostics described in this review should be improved [30, 31, 32]. In order to reduce costs and expand applicability in these areas, it is necessary to study ready-to-use reaction mixtures that are stable at room temperature. Finally, molecular diagnostic methods such as quantitative PCR, differential PCR, and sequencing require equipment, so rapid identification of pathogens in situ may be difficult in resource-limited settings. More productive, automated and portable devices with high sensitivity and specificity, which will contribute to the rapid diagnosis and treatment of infectious diseases worldwide, will be created thanks to the continuous improvement of molecular diagnostic technologies [1, 15, 28, 31, 32, 33, 34].

Conclusion. This review examines the relationship between diagnostic methods and the use of medical resources, as well as the cost of treating patients with acute infectious gastroenteritis in a large outpatient cohort common in many countries.

In the diagnosis of gastrointestinal infections, the use of real-time multiplex PCR revealed shortcomings and increased the detection rates of many pathogens. Our data show how important it is to conduct comprehensive surveillance to monitor the spread of intestinal infections. In this way, the syndrome test panel can provide healthcare professionals with timely and accurate information for more effective treatment and public health interventions.

The high performance, sensitivity and specificity of molecular testing make it possible to quickly diagnose, treat and control gastrointestinal infections. The latest molecular methods are rapidly replacing traditional laboratory methods for the diagnosis of gastrointestinal infectious diseases as a result of advances in technology and available commercial methods.

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