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The Importance of Bacterial Intestinal Infections in Medical Practice and the Prospects of Modern Methods for Detecting Major Pathogenic Infections

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Abstract: The morbidity and death associated with public health are significantly impacted by bacterial infections. Bacterial infections continue to be a huge global economic and social burden despite great advancements in their prevention and treatment. Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species are the six highly virulent multidrug-resistant bacteria that the WHO identified as causing life-threatening illnesses. Together with Salmonella spp., Legionella spp., Escherichia coli, Campylobacter spp. (including C. jejuni and C. coli), Neisseria gonorrhoea, and Clostridium difficile, these microbes are the main contributors to nosocomial infections. In addition to ensuring that the right antibiotic therapy is started early, timely and precise diagnosis of these bacteria is crucial for stopping outbreaks and reducing the development of antibiotic resistance later on. Wastewater-based epidemiology (WBE), a new approach that combines pathogen analysis in wastewater with community infection back-estimation, has enormous potential to enhance existing surveillance systems for infectious disease monitoring and outbreak early warning. However, wastewater presents significant obstacles to the analytical performance of molecular techniques because to its complex matrix. The literature on common pathogenic bacteria in wastewater, biomarker types, molecular techniques for bacterial analysis, and new developments in wastewater analysis were all compiled in this review. To offer guidance for future research, the benefits and drawbacks of various molecular techniques were assessed, and their potential in WBE was explored. To improve epidemiological surveillance of bacterial infections, it is also crucial to have constantly evolving molecular diagnostic tools. Our goal in this review is to go over the latest developments in the diagnosis of bacterial infections using molecular methods based on proteomic and genomic approaches. There is also discussion of the benefits and drawbacks of each of the methods taken into consideration.

Keywords: Antimicrobial resistance, ESKAPE, multidrug-resistant microorganisms, genotyping techniques, epidemiology, antimicrobial resistance.

Introduction. Globally, diseases brought on by human infections pose a serious hazard to public health. The World Health Organization (WHO) estimates that eating contaminated food causes illness in 600 million people (almost one in ten people), 420,000 of whom die annually, resulting in the loss of 33 million healthy lives. In low- and middle-income nations, unsafe food costs them USD 110 billion a year in lost productivity and medical costs. Furthermore, waterborne infections are becoming a global burden, accounting for approximately 2.2 million fatalities annually and nearly USD 12 billion in financial losses. Foodborne and waterborne illnesses put a strain on health care systems and have an impact on economies around the world, which impedes socioeconomic advancement [1-5]. Multidrug-resistant bacteria are rapidly spreading around the world, endangering public health everywhere. According to the World Health Organization (WHO), "ESKAPE," an acronym that identifies these bacteria and their capacity to elude antibiotics' antimicrobial effects, is a group of potentially fatal nosocomial pathogens that are considered critical-priority germs. According to the

WHO, the following are the multidrug-resistant ESKAPE bacteria: *Enterococcus fecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*. These pathogens are the most frequent causes of nosocomial infections, along with *Salmonella spp., Legionella spp., Escherichia coli, Campylobacter spp.* (including *C. jejuni* and *C. coli*), and *Clostridium difficile* [6-9]. Therefore, to facilitate the quick adoption of infection control procedures, sensitive and targeted microorganism detection tests are required. With the advent of hybridization techniques that can detect the presence of specific DNA sequences of interest using short nucleotide base sequences labeled with fluorescent tags, molecular diagnostics have advanced significantly in recent decades. For the quick identification of various infections and the AMR genes linked to them straight from clinical specimens, a variety of commercial assays are available. All of these molecular diagnostic approaches, however, have benefits and drawbacks as compared to traditional approaches, as will be covered below. The 2019 Centers for Disease Control and Prevention (CDC) report found that seven of the 18 pathogens initially listed in the 2013 list were becoming more resistant to critical antibiotics [10-14]. According to the latest paper, carbapenem-resistant Acinetobacter species, which mostly affect hospitalized patients, offer a new and urgent threat by causing pneumonia, bloodstream, wound, and urinary tract infections. *Staphylococcus aureus* is frequently found in the skin, as was previously mentioned, but it also frequently causes infections in individuals who are catheterized. *Pseudomonas aeruginosa* can exacerbate pre-existing lung conditions and cause infections, particularly in hospitalized patients with compromised immune systems. With the exception of tigecycline and colistin, *Enterobacter* species can cause severe nosocomial infections that are resistant to all antibiotics, including blood and urinary tract infections. These are also the last resorts for treating hypervirulent *Klebsiella pneumoniae* that is resistant to carbapenem. One of the main causes of hospital-acquired infections is *Enterococci species* [15-19]. These bacteria can cause more serious infections, such as bloodstream, surgical site, and urinary tract infections, if they travel from the intestines, where they typically dwell innocuously, to other areas of the body. The 2019 CDC study states that in the United States, *E. faecium* was responsible for 5400 fatalities and 54,500 hospitalizations in 2017. The most frequent causes of antibiotic-associated infectious diarrhea are a variety of enteric bacteria, such as *Salmonella species, Campylobacter* and *C. difficile*. Worldwide, *C. difficile* is the organism that causes the most infectious diarrheas and pseudomembranous colitis linked to healthcare settings. The number of nosocomial legionellosis infections has significantly increased in recent years, and the lethality rate for patients in healthcare facilities is higher than 50%. Furthermore, if the bacterium is discovered in hospital facilities like intensive care units—which are intended to intensively monitor and treat patients with life-threatening conditions—the chance of illness rises significantly [20-25].

The main purpose of this review is to conduct a brief analysis of the importance of bacterial intestinal infections in medical practice and the prospects of modern methods for detecting major pathogenic infections.

Finding and Classifying Bacterial Pathogens. Broad-spectrum antibiotics have long been used in clinical settings to address the challenge of detecting bacterial infections and the AMR genes linked to them because there are currently no quick diagnostic techniques for this purpose. In order to avoid chronic infections that may result in complications including wound infections, pneumonia, and bloodstream infections from catheter use that may cause sepsis, it is also essential to identify bacterial pathogens for early, vigorous antibiotic therapy. In order to direct antibiotic therapy and maximize control measures, quick and accurate molecular techniques are required for the identification of extremely virulent drug-resistant bacteria in clinical samples [1-4]. The gold standard for microbial identification typically depends on phenotypic methods, such as automated biochemical testing for identification, antimicrobial susceptibility testing to direct treatment, and the use of enriched and selective culture media for the isolation of pathogenic bacteria. The selection of a microbe-specific medium, the length of time needed for the microbe to spread on the chosen medium, and the time needed to get results are the primary drawbacks of cultivation. On the other hand, laboratory personnel' occupational exposure to infectious agents can be reduced by using molecular diagnostics to directly detect bacteria from clinical specimens. New technologies have been developed that have completely changed the way bacterial infections are diagnosed thanks to advancements in bioinformatics tools and molecular procedures. Discussing the benefits and drawbacks of molecular techniques based on proteomic and genomic technologies for the diagnosis of bacterial infections is the goal of this review [5-14].

Current Developments in Molecular Techniques for Wastewater-Associated Pathogenic Bacteria. Asymptomatic patients are not included in current estimates of the burden of infectious diseases, which are frequently based on severe cases necessitating hospitalization. The examination of biomarkers in raw wastewater serves as the foundation for the newly developed wastewater-based epidemiology (WBE), which is used to back-estimate the public health situation. Wastewater is a complicated concoction of waterborne chemicals and microbes. It includes biological and chemical data that is immediately expelled from our bodies. Urban wastewater is a desirable resource from a monitoring perspective since it offers sample material from a sizable and generally healthy population [3-9]. The WBE method was initially proposed as a possible instrument to assess drug use, both illegal and therapeutic, in a community. Through the detection and measurement of unaltered pharmaceuticals and their human-specific metabolites in wastewater, WBE has developed into a significant tool for measuring both illicit and legal drug consumption. WBE studies also demonstrated that wastewater can indicate a community's risk for infectious diseases in addition to illegal drug usage and diet. The viability of several molecular techniques in wastewater has been confirmed by numerous investigations. In wastewater analysis, sensitive and targeted techniques including PCR, real-time PCR, and DNA sequencing have been used to quickly identify and precisely quantify human pathogens or assess community structure and antibiotic efficacy [11-17].

Methods of Isothermal Amplification and Polymerase Chain Reaction (PCR). High-performance instruments called nucleic-acid-based amplification technologies (NAATs) are employed to quickly and precisely identify pathogen-specific nucleic acids. Mullis created PCR, the first DNA amplification technique, in 1985. It helps create millions of copies of a DNA template from a small number of molecules. In order to create DNA, the PCR method uses thermal cycling, which is a series of heating and cooling cycles of the reaction. Using a DNA polymerase that is stable at high temperatures (hence the method's name), short DNA fragments (referred to as primers) containing sequences complementary to the target region are utilized to accomplish exponential amplification of a target sequence [11-18]. Since its creation, numerous endpoint PCR variants have been created to fast accomplish these two objectives since identifying infections and their resistance mechanisms is a crucial task that needs to be completed as soon as possible in order to stop the spread of antibiotic resistance. Real-time PCR, which is based on PCR techniques, is one of the most popular and clinically used methods for the diagnosis of infectious diseases because of its high sensitivity, specificity, and speed. Using either fluorescent dyes that intercalate unspecifically or probes that are fluorescently tagged specifically, this approach enables real-time monitoring of the target amplification. Only until the probe hybridizes with its complementary target does the fluorescent signal appear, and it is directly correlated with the quantity of PCR amplicons produced. Furthermore, maintaining the RT-PCR device is expensive. Numerous isothermal amplification methods, such as loop-mediated amplification (LAMP), helicase-dependent amplification (HDA), nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification (TMA), have been developed recently as a result of advancements in RT-PCR [5, 7, 9, 10, 19].

Cell surface proteins and bacterial toxins are examples of gene expression products that can be effectively detected and captured using immunology-based techniques. They were extensively employed in the detection, isolation, and enrichment of particular bacteria for wastewater analysis. They work better for identifying and isolating particular pathogens and microbial antigens from wastewater. They can also be used in conjunction with other molecular techniques to obtain a thorough examination of the genome of a target pathogen. They are anticipated to accomplish quick, on-site, real-time, and inexpensive pathogen identification and enumeration in wastewater by integrating with a biosensor platform or paper-based device [11-18]. The analysis of harmful microorganisms in wastewater has demonstrated significant promise using biosensor-based techniques. However, from the perspective of long-term surveillance, they are not cost-effective in wastewater analysis when compared to other molecular approaches, and they are unable to analyze vast quantities of environmental samples, including wastewater. To quickly analyze harmful bacteria in wastewater, paper-based devices work well when paired with a variety of other molecular detection techniques. Their potential as a quick, on-site, affordable, portable, and disposal tool for wastewater has been thoroughly verified. To detect different targets in wastewater, their sensitivity and specificity should be further enhanced [21-26].

Discussion. It is well known that efficient monitoring is essential to the prompt intervention and containment of infectious disease outbreaks, given the growing worries about infectious diseases brought on by harmful bacteria and their resistance to antibiotics. Because of its enormous potential as a population prevalence surveillance system and an early warning tool for disease epidemics, wastewater-based epidemiology has gained popularity. WBE applications are growing to include a variety of pathogens employing various sophisticated molecular techniques designed for wastewater analysis, as a result of the development of molecular techniques for the detection of pathogenic bacteria and related biomarkers. After DNA/RNA extraction processes, PCR-based techniques are widely utilized for the quick identification of harmful bacteria in wastewater due to their high sensitivity [1-7]. Methods such as DNA microarray and sequencing-based methods are suited for the in-depth investigation of bacterial communities and the presence of pathogenic bacteria and antibiotic resistance due to their capability of large-scale parallel examination of the full microbiome. FISH and LAMP are two alternative nucleic acid targeting techniques that are reasonably sensitive, specific, and economical. However, information regarding the activity and contagiousness of different pathogens in wastewater cannot be obtained using nucleic acid targeting techniques. Furthermore, there are still issues with multiplexing microbe identification in a single sample and the requirement for sample preparation. Biosensors are simple to use and do not require skilled workers to identify harmful microorganisms in wastewater [8-12]. Furthermore, due to their speed and affordability, paper-based devices have recently become popular for the detection of pathogenic bacteria in wastewater. Molecular methods have found a variety of uses in wastewater treatment and effluvium (WBE). Nucleic acid-based methods allow for the direct and thorough analysis of the DNA/RNA of wastewater samples, including the detection and quantification of target genes (e.g., species-specific genes, ARGs, and functional genes), the profiling of the entire microbiome in the sample, genome sequencing, and analysis. As a result, nucleic acid-based methods have the most promising potential for the quick and on-site detection of chemicals and microbiomes, which is appropriate for the early warning of infectious disease outbreaks [14-20]. While minimizing the use of antibiotics when they are not required and the related morbidity and medical expenses, prompt detection of serious infections is essential to starting the right treatment as soon as feasible. Because of their capacity to build biofilms and their quick acquisition of AMR genes, ESKAPE pathogens and other major health risks have the biggest influence on infections linked to healthcare. The molecular approach's primary benefits for detecting bacterial pathogens and AMR genes are (1) faster results; (2) direct application of these techniques to clinical samples, which results in a shorter time-to-response; and (3) cost savings due to shorter hospital stays, fewer hospitalizations, and lower risks of co-morbidity and mortality. Despite recent developments in molecular technologies for microbiological diagnostics, no single diagnostic platform can completely satisfy the clinical requirements to (1) improve patient care and treatments, (2) decrease the use of broad-spectrum antibiotics, and (3) attain better clinical [21-27]

Conclusions. It is essential to diagnose dangerous infections quickly in order to start the right treatment as soon as possible. This also helps to minimize the use of antibiotics when they are not needed, as well as the morbidity and medical expenses that come with it. The ESKAPE pathogens and other dangerous health risks have the biggest influence on infections linked to healthcare because of their quick acquisition of AMR genes and capacity to create biofilms.

The primary benefits of the molecular approach to bacterial pathogen and AMR gene detection are (1) faster results; (2) shorter time-to-response due to direct application of these methods to clinical samples; and (3) cost savings through shorter time to appropriate therapy, fewer hospitalizations, and lower risks of co-morbidity and mortality. There isn't a single diagnostic platform that can completely address the clinical need to (1) improve patient care and treatments, (2) decrease the use of broad-spectrum antibiotics, and (3) attain better clinical outcomes, despite recent advancements in molecular technologies in the field of microbiological diagnostics.

Even while molecular techniques have demonstrated significant promise in the identification of harmful bacteria in wastewater, their use in WBE is currently beset by a number of significant obstacles. Other issues with these approaches compared to the analysis of other kinds of samples include the low concentration of harmful bacteria in wastewater and the inhibition of the complex wastewater matrix. It is necessary to increase the low DNA/RNA recovery efficiency of harmful bacteria from wastewater and provide quantitative data. Using proven molecular detection techniques would greatly improve WBE's accuracy and dependability.

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