Asia Pacific Science Press

Asia Pacific Journal of Clinical Medical Research
ISSN(O):3079-8345
ISSN(P):3079-8337
Vol. 1 No.3 (2025)

Research Progress on Detection Technologies for Pseudomonas aeruginosa

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Abstract: Pseudomonas aeruginosa is an opportunistic pathogen widely distributed in the natural environment, which can cause a variety of infections, especially in people with low immunity and high pathogenicity. In recent years, significant progress has been made in the detection technology of Pseudomonas aeruginosa, covering traditional methods, molecular biology techniques, immunological methods and automated detection systems. Traditional methods such as the national standard method and the filter membrane method are easy to operate, but have the problems of long time consuming and limited sensitivity. Molecular biological techniques (such as PCR, gene cloning) and immunological methods (such as ELISA, colloidal gold immunochromatography) have significantly improved the sensitivity and specificity of detection, but they require high equipment and technology, and are expensive. Automated detection systems, such as VITEK 2 Compact and AutoMS 1000 mass spectrometry identification system, are excellent in improving detection efficiency and accuracy, but their high cost and complex operation process limit their wide application. In addition, the resistance of Pseudomonas aeruginosa to bacteriostatic agents further increases the difficulty of detection. In this paper, the development and application of immunological detection technology, molecular biological technology and immunological technology of Pseudomonas aeruginosa were reviewed, and the principles, advantages, disadvantages and research progress of various detection technologies of Pseudomonas aeruginosa were described, and the future development trend was prospected, in order to provide reference for the optimization and development of detection methods of Pseudomonas aeruginosa.

Keywords: Pseudomonas Aeruginosa; Detection Technology; Molecular Biology; Immunology; Automated Detection

Published: Sept 4, 2025

DOI: https://doi.org/10.62177/apjcmr.v1i3.539

Pseudomonas aeruginosa is a Gram-negative bacterium widely distributed in nature. It belongs to the family Pseudomonas, and is commonly found in soil, water, plant surface, and hospital environment. The cell is rod-shaped and its width is about 0.5-1.0um and its length is about 1.5-5.0um. Unlike Salmonella, Pseudomonas aeruginosa does not usually have a capsule, but is able to produce a polysaccharide substance called alginate that forms a biofilm that helps the bacteria survive in harsh environments. Pseudomonas aeruginosa has unipolar flagella, which are motile, and has pili that help it attach to the host cell surface. Pseudomonas aeruginosa is not highly nutrient demanding, can grow under simple carbon and nitrogen source conditions, and can grow in the temperature range of 4 ℃ to 42 ℃ and the pH range of 5.5 to 8.5.

Pseudomonas aeruginosa is an important opportunistic pathogen that mainly infects people with low immunity. It can

cause a variety of infections, including respiratory tract infection, urinary tract infection, wound infection, sepsis, etc. Its pathogenicity is related to A variety of virulence factors produced by it, such as exotoxin A, elastase, phospholipase C, etc., which can damage host cells and tissues and lead to the spread of infection.

1.Detection method

1.1 National Standard Law

GB/T 8538-2008 "Inspection Methods for Drinking Natural mineral Water" stipulates the detection process of Pseudomonas aeruginosa, mainly including sample filtration, selective culture (such as Cetrimide AGAR medium), colony morphology observation and biochemical identification (such as oxidase test, pigment production test, etc.). The results were confirmed by selective medium inhibition of other bacterial growth combined with specific biochemical reactions of Pseudomonas aeruginosa. The determination of the test results was based on whether the bacteria were detected in accordance with the characteristics, and Pseudomonas aeruginosa in drinking natural mineral water should not be detected. In the detection process, it is necessary to strictly aseptic operation, ensure the quality of medium and culture conditions to ensure the accuracy of results[1].

According to the provisions of "National Standard for Food Safety Packaged Drinking Water" (GB 19298-2014), Pseudomonas aeruginosa could not be detected in any of the five packaged samples. Pseudomonas aeruginosa shows a certain tolerance to disinfection methods commonly used in the production of packaged drinking water (such as ozone, ultraviolet light and chlorine dioxide)[2-3], which makes it difficult to completely eliminate possible Pseudomonas aeruginosa in the water, resulting in products that do not meet the standards. Although the content of Pseudomonas aeruginosa detected by enterprises in the early stage of production may be extremely low, due to the long shelf life of packaged drinking water, the bacteria may multiply in large numbers during transportation and sales, and the concentration may even reach 10^4 CFU/mL, which will also lead to unqualified products[4].

In the National standard for Food Safety - Quality Requirements for Food microbiological Test Media and Reagents (GB 4789.28-2013), the detection content of Pseudomonas aeruginosa mainly includes the following aspects: The medium and reagents used for detection should meet the quality requirements in the standard to ensure their applicability and reliability. The medium should have good selectivity and discrimination, which can effectively inhibit the growth of other microorganisms and promote the growth and characteristics of Pseudomonas aeruginosa. The standard specifies the detection steps, including sample processing, inoculation, culture and identification, etc. The commonly used medium is Cetrimide Agar (cetyl trimethylammonium bromide AGAR), which is selective for Pseudomonas aeruginosa[5]. The culture conditions are usually 30-35°C for 24-48 hours. After cultivation, it is necessary to observe the characteristics such as colony morphology and color. Pseudomonas aeruginosa usually shows green or blue-green colonies on Cetrimide Agar, and may produce fluorochrome. After the initial identification, it needs to be further confirmed by biochemical tests (such as oxidase test, pigment production test, etc.), and molecular biological methods (such as PCR) can be used if necessary. According to the test results, whether Pseudomonas aeruginosa was detected in the sample was reported, and the colony count and identification results were recorded. The specific operation should strictly refer to the detailed provisions of GB 4789.28-2013 to ensure the accuracy and reliability of the test.

1.2 Filter membrane method

The detection method adopted the national standard filter membrane method. The water sample was filtered by aseptic operation, so that bacteria were trapped on the filter membrane, and then the filter membrane was attached to Pseudomonas AGAR medium for culture, and the number of typical colonies was counted. The method is simple and sensitive, and is suitable for the detection of Pseudomonas aeruginosa in drinking water.

Mai Miao proposed that sample collection is the key step of detection[6]. When sampling bottled water, it is necessary to disinfect the barrel wall and sampling port with disinfectant water to ensure that the sampling container and apparatus are sterile and avoid cross contamination. The sampling personnel need to sterilize their hands, and shake the water sample sufficiently before sampling to make the bacteria evenly distributed and ensure the uniformity of sampling. The quality control of medium is the core to ensure the accuracy of detection. The selection, preparation and use of media should be

strictly in accordance with the "National standard for Food Safety: Quality Requirements for Culture Media and Reagents for Microbiological Examination of Food" (GB 4789.28-2013) to ensure that the proportion of nutrients and pH value meet the standards. Laboratories should formulate quality control standards and quality control instructions for medium, and strengthen the quality control of compounding water and verification reagents. The preparation method, sterilization method and storage conditions of the medium should be standardized to ensure the stability of its quality.

Special attention should be paid to aseptic operation during inoculation and culture. The filter membrane should be tightly attached to the medium to avoid air bubbles, so as to ensure that Pseudomonas aeruginosa can fully contact the medium and grow. The temperature and humidity of the incubator should be reasonably controlled to provide a suitable growth environment for Pseudomonas aeruginosa and improve the accuracy of strain counting within a suitable environmental range[7]. The accuracy and reliability of Pseudomonas aeruginosa detection can be effectively improved by strictly regulating sample collection, medium quality control and inoculation and culture operations[5].

1.3 Construction and expression of OprF prokaryotic expression vector

The experimental method is based on PCR amplification and gene cloning technology. First, genomic DNA is extracted from Pseudomonas aeruginosa, the target gene fragment is amplified by specific primers, and the amplified products are separated and purified by agarose gel electrophoresis. The purified gene fragment was ligated into the cloning vector and transformed into host cells, such as E. coli DH5α competent cells. Positive clones containing target gene fragments were obtained by antibiotic screening and identification by PCR or enzyme digestion[8]. The positive clones were screened and the recombinant expression plasmid was constructed. The target gene fragment was digested from the cloning vector and ligated into the expression vector (e.g., pET28b). The recombinant plasmids were transformed into competent cells, and positive clones were selected by medium containing antibiotics and further verified by PCR and enzyme digestion. Positive clones were inoculated into the medium containing the inducer to induce expression of the target protein. The expressed cells were separated by ultrasonic fragmentation and centrifugation to obtain the supernatant and precipitate, and then the target protein was purified by affinity chromatography (such as Ni-NTA). Finally, SDS-PAGE was used to analyze the protein expression, and Western blot was used to verify the expression and molecular weight of the target protein.

Wu Qing [9] et al. 's method successfully achieved the cloning, expression, protein purification and identification of OprF gene by PCR, enzyme digestion, SDS-PAGE and Western blot, which provided reliable technical support for Pseudomonas aeruginosa related research.

1.4 Rapid detection based on sugar functionalized magnetic nanoparticle material enrichment and fluorescence method

This assay is based on glycosylfunctionalized magnetic nanoparticles (GNPS) and loaded GNP@FITC functionalized nanofiber membranes for the detection of Pseudomonas aeruginosa in packaged drinking water. 5 mg of sugar-functionalized magnetic nanoparticles was added to 250 mL of packaged drinking water and shaken at 37℃ and 160 r/min for 30 min. The nanoparticles were adsorbed by magnet and the upper bacterial solution was removed (magnetic field separation). Then 20 mL of liquid medium was added and incubated for 6 hours. The membrane was loaded with GNP@FITC functionalized nanofiber membrane, and the fluorescence intensity was measured by fluorescence spectrophotometer after 30 minutes. By comparing the fluorescence intensity ratio (I/I_0) between the initial (I_0) and the (I_0) after 30 minutes of culture (I/I_0) less than 0.7, the Pseudomonas aeruginosa was identified.

In the experiments conducted by Huang Hui et al. [10], the optimal experimental conditions were determined by optimizing the addition amount of glycofunctionalized magnetic nanoparticles, the co-culture time with Pseudomonas aeruginosa, and the co-culture time of the membrane loaded with GNP@FITC functionalized nanofibers and adsorbed bacteria. This method can quickly and sensitively detect Pseudomonas aeruginosa by changing the fluorescence intensity, which provides an efficient technical means for microbial detection of packaged drinking water.

1.5 PCR amplification

Single PCR amplification：Liu Huan et al. used a single PCR amplification technique to construct a PCR reaction system by using the mixed bacterial solution as a template, adding specific primers, DNA polymerase, buffer, and pure water. The

amplification program includes the steps of predenaturation, denaturation, annealing, and extension, and the amplification is completed after multiple rounds of cycling. The amplified products were separated by agarose gel electrophoresis and the amplified bands were analyzed in a gel imaging system to verify the presence of the target gene fragment [11].

Multiplex PCR amplification：Based on the single PCR, the primer volume in the multiplex PCR system was adjusted to 0.125 to 1 μL, and the other reaction components and conditions were consistent with the single PCR. By optimizing the primer ratio and reaction conditions, the specific amplification of multiple target bacteria was achieved at the same time. The amplified products were similarly analyzed by 2% agarose gel electrophoresis and gel imaging system to verify the specificity and sensitivity of the multiplex PCR. Primer specificity was verified by single PCR using the mixed bacterial solution as a template. The concentration of five control bacteria was detected by dilution plate smear counting method, and the concentration of each bacteria in the mixed bacterial solution was calculated. The detection limit of multiplex PCR was analyzed by agarose gel electrophoresis and gel imaging using mixed bacterial solutions with different concentration gradients as templates. The reproducibility of the method was evaluated by repeating 10 times of multiplex PCR using 1×10^5 diluted bacterial mixture as template.

Liu Huan et al. proposed the need for the preparation of simulated samples and the detection of control bacteria [11]. Six sterilized medical protective masks were used to evenly smear 1 ml of 1×10^7 CFU/ml mixed bacterial solution, and placed at room temperature until no visible droplets were observed to prepare simulated samples for controlling bacterial contamination. Test sample solution was prepared according to GB 15979-2002 “Hygienic Standard for Disposable Sanitary products” Appendix B method [12] : weighing (10±1) g sample, cutting into pieces, adding 200 ml sterile physiological saline, and thoroughly mixing. Five ul of the sample solution was used as a multiplex PCR template to detect the five control bacteria in the simulated samples.

1.6 Preparation of monoclonal antibodies and ELISA

In the study of detection methods for Pseudomonas aeruginosa, the preparation of monoclonal antibodies is one of the key steps. BALB/c mice were immunized with the recombinant protein Pa-OprF mixed with Freund’s complete adjuvant at a volume ratio of 1:1. When the serum antibody titer of the mice reached 1:64 000 and the OD value was greater than 0.4, the recombinant protein Pa-OprF was injected intraperitoneally to enhance the immune effect[13]. Three days after immunization, splenocytes from immunized mice were harvested for cell fusion with SP2/0 cells at a ratio of 5:1. The recombinant protein Pa-OprF and inactivated Pseudomonas aeruginosa were used as coating antigens, and the positive hybridoma cell lines that could specifically recognize Pseudomonas aeruginosa were obtained after two subcloning and indirect enzyme-linked immunosorbent assay. The selected positive hybridoma cell lines were injected intraperitoneally into BALB/c mice, and serum was collected and purified to obtain monoclonal antibodies. Using the recombinant protein Pa-OprF as the coating antigen, indirect ELISA was used to detect the titer of monoclonal antibody. S/N>2.1 was judged as positive, and the highest dilution of antibody corresponding to S/N>2.1 was taken as the titer of the antibody[14]. This step provides antibody tools with high specificity and sensitivity for the development of subsequent detection methods.

In this method, in order to evaluate the binding characteristics and specificity of the monoclonal antibody to the antigen, the monoclonal antibody biofilm interference technique and Western Blot were used for detection. Octet Red instrument and Mouse immunoglobulin quantification sensor (AMC) were used to detect the antigenantibody affinity of the purified monoclonal antibodies. Origin 8.5 software was used to process the experimental data and generate the binding dissociation curve to quantitatively analyze the binding kinetic parameters of monoclonal antibodies and antigens. It provides an important basis for the optimization of antibody performance and the improvement of detection methods[15]. To further verify the specificity of the monoclonal antibodies, the method was identified by Western Blot. Pseudomonas aeruginosa was sonicated and the supernatant was collected by centrifugation. After denaturation, the supernatant and the recombinant protein Pa-OprF were separated by SDS-PAGE. After electrophoresis, proteins were transferred to PVDF membrane, blocked with blocking solution and incubated with prepared Pa-2* and Pa-3* monoclonal antibodies as primary antibodies and sheep anti-mouse IgG-HRP as secondary antibodies. Chemiluminescence reagents were used to develop the monoclonal antibody, and the specific recognition ability of the monoclonal antibody to Pseudomonas aeruginosa was verified by the development results.

This step ensures the reliability and accuracy of the antibody in the detection method and provides a solid experimental basis for subsequent applications.

By adopting these methods, Cao Jiamin et al.[16] provided highly specific monoclonal antibodies for the detection of Pseudomonas aeruginosa, and verified the titer, affinity and specificity of the antibodies by ELISA, biofilm interference technology, Western Blot and other methods, laying a solid foundation for the development of efficient and sensitive detection methods for Pseudomonas aeruginosa.

1.7 Colloidal gold immunochromatography

In order to evaluate the sensitivity of colloidal gold immunochromatographic test strips in the detection of Pseudomonas aeruginosa, the test strips were prepared and tested according to the method in literature[17]. Pseudomonas aeruginosa concentrations were diluted to a series of six standard turbidity gradients, and PBS was used as a negative control. The minimum detection limit was determined by observing the positive results of the test strip. This test provides an important basis for the sensitivity of the dipstick in practical applications, ensuring that it can detect low concentrations of Pseudomonas aeruginosa. To verify the specificity of the test strips, Pseudomonas aeruginosa and 10 other common respiratory pathogens, including Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumannii, Mycoplasma pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus salivans and Streptococcus pyogenes, were detected at a concentration of 1.0 McManus unit. PBS was used as a negative control, and its specific recognition ability to Pseudomonas aeruginosa was evaluated by the reaction results of the test strip. This test ensures that the dipstick can accurately distinguish Pseudomonas aeruginosa from other pathogens and avoid cross-reactions in practice. To evaluate the stability and repeatability of the strips, an accelerated aging test was performed. The test strips were placed in 45℃ environment, and random samples were taken at a certain time interval. Pseudomonas aeruginosa at 1.0 McConkey unit concentration was used as positive sample, and PBS was used as negative control. According to the test results, the storage time of the test strip at room temperature was deduced by formula. This test provides reliable data support for the long-term storage and use of the test strip, and ensures its stable detection performance within the validity period[16].

In this study, sensitivity, specificity and stability tests were performed to comprehensively evaluate the potential application of GICA strips in the detection of Pseudomonas aeruginosa. Combined with relevant studies in the literature, this method is rapid, sensitive, specific and stable, which provides strong technical support for the field detection of Pseudomonas aeruginosa.

1.8 VITEK 2 Compact automatic bacterial identification

VITEK 2 Compact is a fully automated microbial identification system that is widely used for rapid identification of bacteria in clinical and environmental Settings. Based on biochemical reactions and microbial metabolic properties, this system can efficiently and accurately identify a variety of bacteria, including Pseudomonas aeruginosa. In this study, VITEK 2 Compact was used to identify the isolated and purified strain 47 and the standard strain ATCC15442.

In the identification process, Shen Peiyao et al.[18] performed Gram staining on strain 47 and standard strain ATCC15442 to confirm that it was Gram-negative bacteria. According to the staining results and colony morphology, the corresponding identification cards were selected for subsequent identification. Single colonies of the strains to be tested were selected and prepared into bacterial suspension with appropriate concentration to ensure the accuracy of identification results. The prepared bacterial suspension was injected into the identification card of VITEK 2 Compact system, and the system automatically performed biochemical reaction detection and data analysis. Identification results were systematically generated by comparing metabolic features in the database. Strain 47 and standard strain ATCC15442 were Pseudomonas aeruginosa, and the identification probability was 97% and 93%, respectively, by VITEK 2 Compact. This result was consistent with the characteristics of the positive strain in the GB 8538-2016 identification criteria, and verified the identity of strain 47 as Pseudomonas aeruginosa.

With its high efficiency, accuracy and high automation, VITEK 2 Compact system significantly shortens the identification time of bacteria. Its rich database covers the metabolic characteristics of a variety of bacteria and can provide high confidence identification results. In this study, the system confirmed the Pseudomonas aeruginosa characteristics of strain 47 with the

results of LAMP assay and culture medium experiments. Combined with LAMP and traditional culture medium methods, VITEK 2 Compact optimized the detection process, improved the detection efficiency, and provided a reliable identification tool for microbiology laboratories.

1.9 Identification by AutoMS 1000 mass spectrometry

AutoMS 1000 mass spectrometry identification system is an efficient microbial identification tool based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology, which is widely used for rapid identification of bacteria in clinical and environmental samples. In this study, AutoMS 1000 was used for the identification of Pseudomonas aeruginosa and carbapenemase detection, which was performed according to the 2022 CLSI operating standards to improve its accuracy and efficiency in microbial detection to a certain extent[19].

In the study of Lu Dan et al.[20], they collected Pseudomonas aeruginosa strains isolated and preserved between January 2022 and October 2023, including 50 carbapenem-resistant Pseudomonas aeruginosa (CRPA) and 30 carbapenem-sensitive Pseudomonas aeruginosa (CSPA). All strains were identified by AutoMS 1000 mass spectrometry identification system and compared with the results obtained by Vitek 2 Compact automatic microbial analysis system. AutoMS 1000 could quickly and accurately identify Pseudomonas aeruginosa by analyzing the protein profile of bacteria. The identification results were consistent with the original stored strains, which verified the high reliability of AUTOMS 1000. AutoMS 1000 can also be used to detect whether Pseudomonas aeruginosa produces carbapenemases. In the study, imipenem solution was prepared with bacterial suspension, and after reacting the strains with imipenem, mass spectrometry analysis was performed using AutoMS 1000. The complex adduct peak (489 m/z) of imipenem and matrix α-cyano-4-hydroxycinnamic acid (HCCA) disappeared when carbapenemase was produced. This method can quickly detect the carbapenemase activity of Pseudomonas aeruginosa, and provide an important basis for clinical treatment. In this study, the AutoMS 1000 mass spectrometry identification system has the characteristics of simple operation, fast detection speed and high accuracy, which can complete bacterial identification and drug resistance detection in a short time. The detection method based on mass spectrometry technology does not require complicated sample pretreatment and significantly shortens the detection cycle. In this study, the identification results of AutoMS 1000 were consistent with the results of K-B method drug susceptibility test, which further verified its reliability in the detection of Pseudomonas aeruginosa.

The AutoMS 1000 mass spectrometry identification system showed significant technical advantages in the identification and carbapenemase detection of Pseudomonas aeruginosa, providing an efficient and reliable tool for the rapid detection of Pseudomonas aeruginosa in clinical and environmental samples. Combined with the relevant studies in the literature, AutoMS 1000 has a broad application prospect in the field of microbial detection, which can significantly improve the detection efficiency and provide strong support for clinical diagnosis and treatment.

1.10 Vitek 2 Compact automatic microbial identification drug sensitivity analyzer

Vitek 2 Compact automatic microbial identification and drug sensitivity analyzer is a highly efficient microbial identification tool based on colorimetric turbidimetry, which is widely used in rapid identification and drug sensitivity testing of bacteria in clinical samples. By analyzing the metabolites and biochemical characteristics of bacteria, the instrument can complete bacterial identification and drug sensitivity analysis in a short time. It has the characteristics of simple operation, high accuracy and good repeatability. In this study, Vitek 2 Compact was used to identify Pseudomonas aeruginosa and other Gram-negative bacteria. By colorimetric turbidimetry, the instrument was used to detect the identification card at three wavelengths every 15 minutes, a total of 16 orientations, and each position was detected three times. Gram-negative bacteria (GN) identification cards can be completed within 3 to 8 hours, depending on the species. The results showed that 73 out of 75 strains of Gram-negative bacteria were correctly identified, and the correct rate was 97.33%, indicating that Vitek 2 Compact has a high accuracy in the identification of Gram-negative bacteria such as Pseudomonas aeruginosa. Vitek 2 Compact was used to read the transmittance values of the reaction Wells of the drug susceptibility test plate, and the sensitivity of bacteria to drugs was determined according to the standard curve.

In the present study by Wu Shu et al.[21], the coincidence rate of drug sensitivity test results of Gram-negative bacteria was 93.33%, indicating that the instrument had high reliability in drug sensitivity test. The results of drug susceptibility test were

divided into coincidence, serious error, major error and general error, and the coincidence rate was as high as 93.33%, which further verified the accuracy of Vitek 2 Compact in the drug susceptibility test of Pseudomonas aeruginosa.

Vitek 2 Compact automatic microbial identification and drug sensitivity analyzer is characterized by its high efficiency, accuracy and high automation, which can complete bacterial identification and drug sensitivity test in a short time. Based on the detection principle of colorimetric turbidimetry, it can quickly analyze the metabolic characteristics of bacteria and significantly shorten the detection time of traditional methods. In this study, Vitek 2 Compact identification and drug susceptibility test results showed high accuracy and reliability, which provides an important basis for clinical diagnosis and treatment. The analyzer showed significant technical advantages in the identification and drug sensitivity test of Pseudomonas aeruginosa, providing an efficient and reliable tool for the rapid detection of clinical samples. Combined with the relevant literature, Vitek 2 Compact has a broad application prospect in the field of microbial detection, which can significantly improve the detection efficiency and provide strong support for clinical diagnosis and treatment.

2.Discussion

The detection technology of Pseudomonas aeruginosa has made significant progress in recent years, covering a variety of methods from traditional culture methods to modern molecular biology and immunological techniques. Although these techniques have their own advantages in sensitivity, specificity, and detection speed, they also face some common challenges and limitations. Although the traditional national standard method and the filter membrane method are simple to operate and low cost, their detection cycle is long, usually takes several days, and the operation skills of the experimental personnel are high, which may have the problem of insufficient sensitivity when detecting low concentrations of bacteria. Despite their widespread use in laboratory Settings, these methods are less efficient in field applications where rapid detection is required.

Detection methods based on molecular biology, such as PCR and LAMP technology, have high sensitivity and specificity, and can be completed in a short time. However, these methods require high equipment and reagents, are relatively expensive, and require high operational skills of the experimental personnel. When PCR and LAMP techniques detect complex samples, they may be interfered by inhibitors in the samples, which may affect the accuracy of the detection results. Immunological methods, such as ELISA and colloidal gold immunochromatography, are fast, sensitive and specific, and are suitable for rapid detection in the field. However, the stability and reproducibility of these methods still need to be further optimized, especially in the process of long-term storage and use, false positive or false negative results may occur. However, the high cost of development and production of immunological methods limits their promotion in large-scale applications.

Automated detection systems, such as VITEK 2 Compact and AutoMS 1000, can rapidly and accurately identify Pseudomonas aeruginosa and assess its drug susceptibility by combining biochemical reaction and mass spectrometry. These systems have demonstrated significant advantages in clinical diagnostics, but their high equipment costs and [] maintenance costs limit their use in resource-limited Settings. In conclusion, although the detection technology of Pseudomonas aeruginosa is improving, there is still a need for further research and development of low-cost, high-throughput, and suitable for rapid detection in the field. Future research should focus on improving the sensitivity, specificity and stability of detection methods while reducing the cost of detection to meet the needs of different application scenarios. Through multidisciplinary cooperation and technological innovation, the detection technology of P. aeruginosa is expected to play a greater role in the fields of clinical diagnosis, food safety and environmental monitoring.

Although the detection technology of Pseudomonas aeruginosa has made significant progress, there are still some problems to be solved. Although traditional detection methods such as national standard method and filter membrane method are simple to operate, they are time-consuming and limited in sensitivity, which are difficult to meet the needs of rapid detection. Although molecular biological techniques (such as PCR, gene cloning, etc.) and immunological methods (such as ELISA, colloidal gold immunochromatography, etc.) have improved the sensitivity and specificity of detection, these methods require high equipment and technology, and are expensive, limiting their application in resource-limited Settings. The tolerance of Pseudomonas aeruginosa to disinfectants and its potential ability to reproduce in packaged drinking water make it difficult to completely eliminate the risk of contamination with existing detection methods. Although automated detection systems, such as VITEK 2 Compact and AutoMS 1000 mass spectrometry identification system, have significantly improved the detection

efficiency and accuracy, their wide application is still limited by the high equipment cost and complex operation process.

In the future, the development of Pseudomonas aeruginosa detection technology should focus on the following aspects: the development of more rapid, sensitive and low-cost detection methods, such as fluorescence detection technology based on nanomaterials and portable detection equipment, to meet the needs of rapid on-site detection; Further optimization of molecular biology and immunology techniques to improve their specificity and stability, while reducing the cost of detection; To strengthen the research on the drug resistance mechanism of Pseudomonas aeruginosa and develop new detection methods for drug-resistant strains. Promote the popularization of automated testing systems, and make them available in more laboratories and medical institutions through technical improvement and cost control. Interdisciplinary cooperation and technology integration will also provide new ideas and solutions for the development of Pseudomonas aeruginosa detection technology, and ultimately achieve the goal of more efficient and accurate detection.

Funding

1.College Students' Innovation and Entrepreneurship Training Program Project(X202511049398).

2.College Students' Innovation and Entrepreneurship Training Program Project(X202511049201).

3.College Students' Innovation and Entrepreneurship Training Program Project(D202504071303298456).

4.Hainan Vocational University of Science and Technology University-Level Scientific Research Funding Project(HKKY2024-87).

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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