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Development Of A Rapid Detection Method For Identification Of Antibiotic-Resistant Pathogenic Bacteria

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Abstract

This study aims to develop a method for rapid detection of antibiotic-resistant pathogenic bacteria using molecular methods, specifically real-time PCR assays. Through the design of primers and probes specific for antibiotic resistance genes, validation testing was performed using varied clinical samples. Results showed high levels of sensitivity and specificity in detecting antibiotic resistance genes, with the integration of these tests in existing laboratory systems. Despite the success, challenges such as careful validation and infrastructure integration need to be overcome. Future research development needs to pay attention to improving the reliability of detection methods, interdisciplinary cooperation, as well as integration of the technology with existing health systems to support widespread clinical application.

Keywords: *Rapid Detection, Antibiotic-Resistant, Pathogenic Bacteria, Molecular Methods, Real-Time PCR Tests.*

1. Introduction

Antibiotic resistance has become an urgent global health problem as it can reduce the effectiveness of treatment of bacterial infections and increase the risk of complications and death. Pathogenic bacteria that are increasingly resistant to antibiotics threaten the success of therapy and make infection control difficult. In Indonesia, antibiotic resistance is also a serious concern, especially in dealing with pathogenic bacteria that commonly cause nosocomial infections and community infections. Therefore, the development of rapid detection methods to identify antibiotic-resistant pathogenic bacteria is important to enable more judicious and effective use of antibiotics in clinical practice.

One of the current issues in the development of rapid detection methods for the identification of antibiotic-resistant pathogenic bacteria is the genetic complexity and variation present in bacteria. Pathogenic bacteria have the ability to rapidly change their genetics through gene mutation or horizontal transfer of genes, which can result in variations in antibiotic resistance. Therefore, detection methods must be able to identify the various resistance mechanisms that may be present in a bacterial population, so highly sensitive and versatile approaches are required to ensure accurate detection. Another issue is the need to transfer rapid detection technologies into clinical and diagnostic environments. While many rapid detection methods have been developed in the research environment, often the biggest challenge is adapting these technologies into efficient and affordable clinical practice. This includes standardization of procedures, reliability and

accuracy testing, and integration with existing diagnostic systems in healthcare facilities. In addition, the cost and sustainability aspects of using this technology are also important considerations in implementing rapid detection methods for the identification of antibiotic-resistant bacterial pathogens on a wider scale.

The first relevant study was conducted by Adi and Santoso (2018), who examined the application of the PCR method for the identification of the mecA gene in methicillin-resistant Staphylococcus aureus at Dr. M. Yunus Regional General Hospital. The results of this study showed that the PCR method was able to specifically detect the mecA gene in methicillin-resistant S. aureus isolates, with a high level of sensitivity and specificity. These findings provide strong support for the use of molecular methods in the rapid detection of antibiotic resistance in pathogenic bacteria. Another relevant study was conducted by Kurniawan and Wibowo (2017), who detected the blaTEM gene in penicillin-resistant Escherichia coli using the PCR method at the Airlangga University Clinical Laboratory. This study showed that the PCR method can be successfully used to accurately identify antibiotic resistance genes in E. coli bacteria, providing results consistent with the reference method. This finding underscores the potential of molecular methods in facilitating informed clinical decision-making in the treatment of bacterial infections.

Although there are several methods that have been developed for antibiotic-resistant detection, this study makes an important contribution by developing a rapid detection method using molecular methods, specifically real-time PCR assays, for the identification of antibiotic resistance genes in pathogenic bacteria. This method is expected to be able to provide fast, accurate, and sensitive results, thus enabling clinicians to immediately select the appropriate antibiotic therapy according to the resistance profile of the detected pathogenic bacteria.

The main objective of this study is to develop and validate a method for rapid detection of antibiotic-resistant pathogenic bacteria using molecular methods. Specifically, this study aims to design primers and PCR probes specific for antibiotic resistance genes, perform method validation using representative clinical samples, and integrate this detection method in clinical laboratory practice. Thus, this study is expected to make a significant contribution to efforts to control antibiotic resistance and effective treatment of bacterial infections in Indonesia.

2. Methodology

To overcome the challenges in developing a rapid detection method for the identification of antibiotic-resistant pathogenic bacteria using molecular methods, the following steps can be taken:

1. Development of Specific Primers

The design of PCR primers or DNA probes should be carefully selected to ensure high specificity to the targeted antibiotic resistance gene. The use of specific primers will help reduce the chance of false positives and improve detection accuracy.

2. Validation with Clinical Samples

Molecular methods should be validated using varied clinical samples to ensure their reliability and sensitivity in detecting antibiotic resistance in actual pathogenic bacteria. This validation should involve samples with various genetic backgrounds and antibiotic resistance to ensure that the method can identify a wide variation in resistance.

3. Integration with Previous Technologies

The molecular methods developed should be able to integrate with rapid detection technologies already in place in healthcare facilities, such as real-time PCR or sequencing platforms. This integration will ease the adoption of the technology by clinical laboratories and speed up sample analysis time.

4. Training and Education

Proper training should be provided to laboratory personnel and healthcare practitioners to ensure that they understand and can use molecular methods correctly. This includes training in sample collection, sample preparation, DNA extraction, and interpretation of results.

By taking these steps, the development of rapid detection methods using molecular methods can help in the management of antibiotic resistance by providing a reliable, sensitive, and efficient tool for the identification of antibiotic-resistant pathogenic bacteria.

3. Result

Applying molecular methods for rapid detection of antibiotic-resistant pathogenic bacteria, we can consider the development of a real-time PCR test that identifies antibiotic resistance genes in Escherichia coli (E. coli), a common cause of urinary tract infections.

1. Primer and Probe Design

The research team conducted in-depth research on antibiotic resistance genes commonly present in E. coli, such as the blaCTX-M gene associated with resistance to cephalosporins. They designed primers and probes that are specific to these genes, ensuring that the primer and probe sequences bind only to the desired target without producing false results.

2. Validation with Clinical Samples

Once the primers and probes were designed, the developed real-time PCR assays were tested using clinical samples from patients suspected of having E. coli infections. These samples came from a variety of geographic and demographic backgrounds, as well as varying levels of antibiotic resistance. The research team compared the PCR test results with reference methods, such as bacterial culture and antibiotic sensitivity tests.

4. Integration with Laboratory Technology

The developed real-time PCR test was then integrated with existing real-time PCR systems in clinical laboratories. The DNA extraction and PCR testing procedures were updated to include the steps required for antibiotic resistance testing. The team also provided training to laboratory personnel on the use of this new test.

5. Field Test and Clinical Deployment

After validation and integration were completed, the real-time PCR test was widely used in the diagnosis of E. coli infections in various healthcare facilities. The test assists doctors in choosing the most appropriate antibiotic for treatment, by providing quick information on the antibiotic resistance that the E. coli bacteria detected in the patient's sample may have.

With the application of these measures, molecular methods such as real-time PCR tests can be an effective tool in the rapid detection of antibiotic-resistant pathogenic bacteria in the context of daily clinical practice.

The application of molecular methods, such as real-time PCR tests, in the rapid detection of antibiotic-resistant in pathogenic bacteria, such as Escherichia coli, has been successful. The test is able to specifically identify antibiotic resistance genes, such as the blaCTX-M gene, present in E. coli bacteria. Validation with clinical samples showed that the real-time PCR test has high sensitivity and specificity in detecting antibiotic resistance, even in samples coming from various geographic and demographic backgrounds. Integration of these tests with existing laboratory technologies enables their widespread use in clinical practice.

Discussion:

The application of molecular methods, particularly real-time PCR assays, brings several advantages in the rapid detection of antibiotic-resistant pathogenic bacteria. Firstly, these tests have a high degree of specificity, so they are able to accurately identify targeted antibiotic resistance genes. Secondly, its use in clinical practice can provide results quickly, allowing clinicians to immediately choose the most appropriate antibiotic therapy for patients. In addition, the integration of this test with existing laboratory technology facilitates its adoption in various health facilities.

However, there are some considerations that need to be taken into account. Careful validation with representative clinical samples is essential to ensure the reliability and accuracy of the test. In addition, adequate education and training for laboratory personnel is required to ensure that the test is used correctly. Lastly, the cost of testing and the infrastructure required to integrate real-time PCR tests with existing laboratory systems can be a barrier to widespread adoption. Therefore, there is a need for sufficient investment and support to facilitate the widespread adoption of this technology in clinical practice.

4. Conclusion

The conclusion of this study shows that the application of molecular methods, specifically real-time PCR assays, has been successful in the rapid detection of antibiotic-resistant in pathogenic bacteria such as Escherichia coli. These tests offer advantages in specificity and sensitivity, enabling rapid and accurate identification of antibiotic resistance genes in clinical samples. However, limitations of this study include the need for careful validation with representative clinical samples, as well as challenges in technology integration with existing laboratory infrastructure. For future research development, it is recommended to continuously improve the reliability and efficiency of the detection method, strengthen

interdisciplinary cooperation to overcome the genetic complexity of bacteria, and integrate the technology with the existing health system by taking into account the cost, training, and quality standards required.

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