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Multiplex Antimicrobial Resistance Gene Detection for Urinary Tract Infection Antibiotic Guidance

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Abstract

The identification of antimicrobial resistance markers in urinary tract infections could lead to a more targeted approach to UTI diagnosis and treatment, reducing overall public health burdens. We describe a molecular assay as a diagnostic tool for antibiotic resistance characterization in order to promote faster antibiotic regimen diagnosis when compared to standard microbiology techniques. Targeted antibiotic use for pathogenic infections remains a primary goal for effective antibiotic treatment protocols and lowering the overall public health burden. Rapid identification of the pathogen causing the infection and harbouring the antibiotic resistance gene is also an important area of research for antibiotic appropriation and stewardship.

Keywords: Antibiotic resistance • Urinary tract infections • Multiplex PCR

Introduction

A genetic diagnostic method for rapid molecular characterization of antibiotic resistance genes is required to reduce inappropriate antibiotic exposure while improving the overall treatment model for urinary tract infections. The goal of this study is to show the logical viability of real-time molecular diagnostics for early detection, active surveillance, and overall targeted antibiotic stratification as an in vitro rapid and comprehensive tool for assessing proper antibiotic stewardship in UTIs. In this paper, we describe a multiplex real-time fluorescence polymerase chain reaction for probe-based detection of the top 24 antibiotic resistance genes with specific relationships to target molecular drug classes and antibiotics. Multiplexed molecular analysis enables rapid testing while shifting the diagnostic detection paradigm from monocentric infections to polymicrobial infections.

Urinary tract infections are common bacterial infections that cost the health-care system billions of dollars each year and affect an estimated 150 million people worldwide. In 2007, there were an estimated 10.5 million UTI-related hospital visits in the United States alone, with nearly 21% resulting in hospital emergency department visits, compared to 2018 estimates of approximately 3 million US hospitalisations for complicated UTIs alone. UTIs are one of the most common clinical conditions for which antibiotics are prescribed. Antibiotic overuse and prolonged therapeutic duration are linked to antibiotic resistance and poor patient outcomes like hospital mortality, high readmission rates, Clostridium difficile infections, and antimicrobial adverse drug events [1].

Literature Review

According to the Centres for Disease Control and Prevention (CDC), up to 50% of all antibiotics prescribed for infectious diseases are administered incorrectly. According to the CDC, at least 2 million people in the United States are infected with antibiotic-resistant bacteria, with approximately 23,000 people dying each year. Antibiotics are commonly used to treat UTIs, but with the rising rate of antibiotic resistance, it is critical to rigorously test UTIs for the presence of UTIs in order to accurately inform clinicians when prescribing targeted antibiotic therapies. Dipstick tests and urine culture on blood agar plates are currently used to isolate a specific pathogen and determine antibiotic susceptibility testing and minimum inhibitory concentration profiles for UTIs.

Culture methods have a limited ability to detect many microorganisms and may overlook fastidious and non-aerobic organisms, as well as slow growing microbes and many Gram-positive organisms. While it has been demonstrated that modifying culture methods with improved growth conditions can significantly increase uropathogen detection rates in symptomatic patients, the process is time consuming. PCR-based methods have gained popularity in the field because they produce more accurate and timely results than traditional culture methods. A recent study compared multiplex PCR-based molecular testing to standard urine analysis across 582 specimens and discovered that the results of the two methods agreed in some cases. Single-organism infections are usually treatable with an antibiotic regimen based on antibiotic susceptibility tests.

Many polymicrobial infections go undetected because clinical microbiology techniques do not report organisms isolated from urine in mixed cultures unless there is a significant count of a predominant microorganism. The simultaneous detection of a greater number of pathogens may improve the outcome of UTIs. When determining diagnostic efficacies, we previously mentioned that UTIs have polymicrobial characteristics that may be more representative of clinical disease than single isolate urine culture methods. Infections with multiple microbes have a higher pathogenic potential than monomicrobial culture samples. As a result, traditional microbiology culture may be lacking in the sensitivity required to correctly diagnose UTIs with antibiotic resistance profiles. We present a multiplexed nucleic acid detection diagnostic for ABR gene identification and characterization [2,3].

The most common diagnostic method for identifying UTIs is monocentric uropathogen isolation via urine culture. UTIs have polymicrobial infections combined with heterogeneity in antibiotic resistance genes, allowing for persistent infections that culture may miss due to restrictive growth mediums and fastidious microorganisms. Persistent infections can be observed over time and after therapy, implying an active bacterial evolutionary process in which polymicrobial infections facilitate antibiotic resistance gene transfer. When compared to multiplex molecular panels, urine culture can identify a limited set of pathogens but is likely to miss polymicrobial infections and cannot identify antibiotic resistance genes within a critical diagnostic window. Such UTI-related bacterial evolution can result in antimicrobial resistance and relapse, posing challenges to current UTI diagnostic standards [4].

Discussion

In this paper, we describe a rapid, molecular multiplexed characterization of uropathogens with antibiotic resistance gene identification, which is critical for

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understanding the polymicrobial nature of UTIs and shifting the diagnostic paradigm towards molecular methods. While antibiotic sensitivity testing has traditionally provided single pathogenic infectious states for a limited number of organisms, urine culture does not provide the multivariable resolution required to understand the polymicrobial interactions in UTIs that promote persistent infection, antibiotic resistance, and disease progression.

In this study, we used samples from the CDC and FDA AR Isolate Banks, which contain bacterial isolates that are frequently used to learn more about known and novel resistance mechanisms in order to develop novel diagnostic methods and therapeutics. The availability of their genetic and phenotypic characteristics to compare with the results of the proposed multiplex qPCR in order to determine concordance between standard microbiological techniques and rapid molecular diagnostics is an advantage of using these samples.

Standardised Multiplex Reaction Model: A standardised reaction model is established for the multiplexing of four interrogated amplicon targets using 5′ nuclease real-time PCR assays with 12 oligonucleotides in a real-time PCR reaction. Each reaction employs four distinct fluorophores to detect 5′ nuclease probes during reaction cycles. For the 4-plex reactions, a standardised custom multiplex optimised master mix is used. Using fluorescence readings and cycle threshold values, a single reaction can identify four distinct targets within a single well/compartment. The combination of this model allows for multiple reactions targeting specific interrogated amplicon sequences in a scaled format, allowing for UTI diagnostics with 24 antimicrobial resistance markers grouped into 6 reactions [5,6].

Conclusion

Finally, cost savings can be realised when comparing culture vs. multiplex molecular methods. A trained and certified laboratory technician, as well as culturing reagents and materials such as agar plates, bacterial broth, glassware/plastic ware, incubators, and other machines, are all required for urine cultures. A technician, PCR reagents such as primers and probes, PCR plates, and a well-maintained PCR machine are all costs associated with a multiplex PCR-based assay. Based on the costs of PCR-based rapid HIV tests as a comparator, the proposed multiplex molecular ABR assay is estimated.

Acknowledgement

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Conflict of Interest

None.

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