УДК: 577.21; 577.15

Original article

ADVANCEMENT IN *STAPHYLOCOCCUS AUREUS* DETECTION USING A RPA-CRISPR-CAS12A FLUORESCENT ASSAY TECHNOLOGY

Zeinulin Murat[1], Amanzholova Meruyert[2], Shaizadinova Aisha[3], Abeldenov Sailau[1] *

[1] S.Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[2] National Center for Biotechnology,Astana,010000, Kazakhstan
[3] al-Farabi Kazakh National University, Almaty, Kazakhstan
** abeldenov@gmail.com*

ABSTRACT

This study explores the utility of CRISPR/Cas-based diagnostics for the precise identification of *Staphylococcus aureus* (*S. aureus*), particularly when integrated with recombinase polymerase amplification (RPA). *S. aureus*, a versatile Gram-positive bacterium, presents significant challenges due to its antibiotic resistance and its capacity to cause a variety of infections. While existing diagnostic methods provide accuracy, they often involve time-consuming procedures and require specialized equipment. Our research introduces a promising approach that leverages the specificity of CRISPR/Cas technology to target genetic sequences, enabling the precise detection of *S. aureus*. Through the combination of CRISPR/Cas and RPA, we expedite the detection process, allowing for rapid and highly sensitive identification of the pathogen's genetic material. Notably, our investigation demonstrates excellent results for the *gsa* and *arcC* genes, affirming the potential of this method as a dependable and highly sensitive molecular diagnostic tool. When compared to traditional diagnostic methods such as microbiology and PCR, our approach offers advantages in terms of speed and sensitivity. Additionally, the inclusion of RPA as a pre-amplification step enhances the accuracy of nucleic acid detection. While our study represents an initial step in technological advancement, it highlights the potential of CRISPR/Cas-based diagnostics to revolutionize disease detection and management. The RPA-CRISPR/Cas has the potential for detecting a wide range of diseases and pathogens, with broad applications in both medical and scientific fields.

Key words: *Staphylococcus aureus*, diagnostic, CRISPR/Cas, RPA, Cas12a, GSA gene, ArcC gene.

INTRODUCTION

S. aureus, is a versatile and clinically significant bacterium that has intrigued scientists and healthcare professionals for decades [1]. This Gram-positive, round-shaped bacterium is a natural inhabitant of the human body, typically residing in the nasal passages and on the skin. In most cases, it coexists harmlessly with its human host. Perhaps one of the most concerning aspects of *S. aureus* is its ability to develop resistance to antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a notorious example. MRSA strains have acquired resistance to numerous antibiotics, making them challenging to treat. These resistant strains have become a significant public health concern, especially in healthcare settings [2]. However, *S. aureus* can also shift its behavior dramatically, transforming from a commensal into a pathogen responsible for a wide range of infections, some of which can be life-threatening [3]. Its ability to cause disease is attributed to various virulence factors and its adaptability. Here are some of the primary causes of *S. aureus* infections: respiratory infections like pneumonia, skin and soft tissue infections, bloodstream infections or bacteremia, infections in bones (osteomyelitis) and joints (septic arthritis), heart infections (endocarditis), toxic shock syndrome, food poisoning [4].

Thus, accurate *S. aureus* identification and diagnostic is crucial for effective patient care and infection control. Diagnostic methods include clinical assessment, Gram staining, culture, biochemical tests, molecular diagnostics, and imaging. Gram staining provides rapid, but nonspecific information. Culture is a gold standard, but time-consuming and take from 2 to 10 days [5]. Molecular diagnostics like PCR offer rapid, accurate detection, but requires expensive equipment and highly qualified personnel [6].

In light of this, there is a need to develop new diagnostic methods that combine accessibility, cost-effectiveness and speed without compromising accuracy and sensitivity. Thus, one of the most promising directions in molecular diagnostics of infectious diseases is the use of CRISPR-based diagnostic [7]. CRISPR-Cas technology, initially originated from its genome-editing capabilities, has transcended its original purpose and is now at the forefront of diagnostics. By utilizing the exquisite specificity of CRISPR-Cas systems in targeting genetic sequences, it becomes possible to detect pathogen's genetic targets with exceptional precision. This technology, when integrated with recombinase polymerase amplification (RPA) [8], amplifies the targets, enabling rapid and sensitive identification of the pathogen's genetic material.

This article is devoted to *S. aureus* identification based on CRISPR/Cas with isothermal amplification RPA. The technology operates based on the identification of the protospacer adjacent motif (PAM) motif situated on the amplified DNA region. When the Cas enzyme recognizes this motif, it forms a complex with the target through the crRNA [9]. Subsequently, cis activity occurs, resulting in the cleavage of the DNA. Detecting the target induces structural alterations in the Cas enzyme, leading to non-sequence-specific trans activity that involves the cleavage of single-stranded DNA [10]. This unique property serves as a foundation for diagnostic purposes, where detection can be achieved, for example, by observing the emission of a fluorescent signal. From point-of-care testing to surveillance in healthcare settings, RPA-CRISPR/Cas diagnostics holds significant promise in enhancing ability to diagnose *S. aureus* infections. By doing this, it provides a significant contribution to the broader initiatives focused on disease management and prevention.

Table 1. Oligonucleotides

Method	Oligonucleotide	Sequence (5'→3′)
RPA	ArcC-Fw	CCGCTTCAACACCTTCATAGGTATTTTCTT
	ArcC-Rv	TAAAGAAGATGCAGGACGTGGTTATAGAAA
	GSA-Fw	GCTAGACCTTTAGATAAAGCTGTAATGGCA
	GSA-Rv	CGGAATTACGTCAGTATTTATTTTCTGGGG
In vitro transcription	MbCas12a-crRNA-ArcC-compl	**GTATAGATTGAGGTAGTGGTGACG**ATCTACAAACAGTAGAAATTCCCTATAGTGAGTCGTATTAGAATT
	MbCas12a-crRNA-GSA-compl	**CAGTAGATAATACAACATTGTTAT**ATCTACAAACAGTAGAAATTCCCTATAGTGAGTCGTATTAGAATT
	crRNA-SHORT	AATTCTAATACGACTCACTATAGGG
Fluorescence	ssDNA Reporter	FAM-TTATT-BHQ-1

MATERIAL AND METHODS

Oligonucleotides

The following oligonucleotides were used in the work (Table 1).

crRNA Design

To generate crRNA, we accessed genetic data from various sequences associated with *S. aureus* ArcC and GSA, which were accessible through the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/genome). DNA fragments extracted from the chromosomal sequences (GenBank) of these genes served as the primary DNA templates. We opted for segments within these genes, ranging in size from 200 to 400 nucleotides, to serve as the basis for amplification. The crRNA design centered on identifying regions proximate to the protospacer adjacent motif (PAM). In consideration of the specificity of the amplification products, we selected a single site for each gene.

Synthesis of crRNAs

To synthesize crRNAs, we employed the HiScribe™ T7 High Yield RNA Synthesis Kit from New England Biolabs. In order to create the DNA templates for subsequent *in vitro* transcription, we utilized synthetic DNA oligonucleotides of varying lengths, each containing a double-stranded T7 promoter region located before the transcription sequence. The minimal T7 promoter sequence employed was 5'-TAATACGACTCACTATAGGG. To construct the template, a short oligonucleotide (10 μM) was combined with its complementary oligonucleotide (10 μM) through a process involving heating to 75°C for 2 minutes, followed by gradual cooling to room temperature over 30 minutes.

The resulting duplex oligonucleotide then functioned as the foundation for the *in vitro* transcription process. The transcribed RNA underwent purification utilizing the Monarch® RNA Cleanup Kit from New England Biolabs, based on the manufacturer's guidelines. This purification included treatment with DNase I to eliminate any remaining template DNA. The purified RNA was quantified using a Nanodrop spectrophotometer manufactured by Thermo Scientific, and it was subsequently diluted to the appropriate working concentrations in diethyl pyrocarbonate (DEPC)-treated water to ensure the absence of nucleases. The eluted RNA was either employed immediately or stored at -80°C for future use.

Positive control inserts

The construction of a plasmid containing the positive control followed this procedure: the genes ArcC and GSA were amplified using Phusion DNA polymerase through polymerase chain reaction (PCR). The PCR products, measuring 220 and 430 base pairs in size, respectively, were verified as positive and subsequently integrated into a genetic construct with the aid of the CloneJET PCR Cloning Kit from Thermo Scientific. The resulting plasmid underwent sequencing to verify the absence or presence of mutations. This positive control played a pivotal role in fine-tuning the recombinase polymerase amplification reaction and evaluating the analysis's sensitivity.

RPA reaction conditions

The recombinase polymerase amplification (RPA) was performed in a final volume of 10 μL. Initially, 2.4 μL of each 10 μM forward (Fw) and reverse (Rv) primers were mixed, followed by the addition of 13.5 μL of diethyl pyrocarbonate (DEPC)-treated water and 29.5 μL of rehydration buffer. After mixing, the master mix was transferred to tubes containing lyophilized RPA enzymes, and 2.5 μL of 280 μM magnesium acetate (MgOAC) was added. Subsequently, 9 μL of the master mix was dispensed, and 1 μL of template DNA was added. The reaction was carried out at an isothermal temperature of 39°C for 30 minutes.

RPA-CRISPR/Cas12a fluorescence assay

To assemble the MbCas12a and crRNA complexes, 1 μM of MbCas12a (obtained from laboratory stock) and 1 μM of crRNA were incubated at 25°C for 15 min. The trans-cleavage reaction of Cas12a was conducted in a 30 μl volume containing NEB 2.1 buffer (New England Biolabs), 100 nM crRNA, 100 nM MbCas12a, 0.5 μM reporter molecule, and 3 μl of RPA amplification products. The reaction mixture was incubated at 37 °C for 2 hour. The reaction results were visualized using a Vilber Lourmat transilluminator (France) with the naked eye at a wavelength of 320 nm. Images were captured using a smartphone camera and saved for further analysis.

Evaluation of limit of detection and specificity of RPA-Cas12a detection

To assess the sensitivity of the RPA analysis, we conducted reactions using ten-fold serial dilutions of both genomic DNA and plasmid DNA. Plasmid DNA containing the sequence of the ArcC, GSA gene fragments was diluted from 3.06×, 4.67×to 3.06×, 4.67× copies per reaction, respectively.

Reagents

All reagents were from New England Biolabs, Sigma and Thermo Scientific in the molecular biology category. The Cas12a enzyme from *Moraxella bovis* was produced in our own laboratory.

RESULTS

The principle of RPA-CRISPR/Cas12a Assay

Recombinase Polymerase Amplification (RPA) is an alternative to the Polymerase Chain Reaction (PCR). RPA technology enables the amplification of genetic material in less than 15 minutes, and the reaction occurs under isothermal conditions.

Diagnostics based on Cas12a utilizes the CRISPR/Cas system for specific detection of nucleic acids. Cas12a, an RNA-guided endonuclease, when activated by a specific DNA target sequence, cleaves nearby single-stranded DNA (ssDNA) molecules, resulting in the release of a fluorescent signal. However, Cas12a is sensitive to the quantity of DNA targets in the sample, and therefore, a pre-amplification stage may be required to enhance the sensitivity of the analysis. Using RPA as a method for pre-amplification in Cas12a-based diagnostics offers several advantages.

RPA is a fast and straightforward method that does not require specialized equipment or extensive expertise. Furthermore, RPA can amplify targets from very small amounts of DNA targets, making it a highly sensitive method. Combining RPA with Cas12a-based diagnostics can increase the sensitivity of the analysis and minimize the occurrence of false-negative results.

The RPA amplification products should contain the target sequence and the PAM region. Specifically, for this study, sequences specific to the *S. aureus* genes *arcC* and *gsa*, which encode bacterial carbamate kinase and glutamate synthetase, were selected as targets. These genes are commonly used as diagnostic markers for identifying *Staphylococcus aureus*. We chose one target each (crRNA-PAM) with identical PAM sequences (TTTG) for the *arcC* gene and PAM (GTTG) for the *gsa* gene to ensure a reliable comparison of enzyme activity and eliminate potential differences in the kinetics of Cas12a-crRNA/target complex formation.

Evaluation of limit of detection

In Figure 1A, we present the amplification of RPA products derived from plasmid dilutions containing a positive control. Meanwhile, Figure 1B exhibits the detection of trans-cleavage activity conducted by Cas12a, utilizing RPA products as the target molecules. The outcomes of agarose gel electrophoresis reveal that the RPA reaction persists even down to a plasmid DNA dilution of copies. The Figure 1B distinctly exhibits a signal that surpasses the background level, indicative of the successful identification of the target genetic sequence. The established limit of detection, quantified at copies per reaction, strongly suggests that the RPA-Cas12a method possesses a remarkable sensitivity in detecting the *arcC* gene locus.

Figure 1C portrays the amplification of RPA products extracted from plasmid dilutions accompanied by a positive control. On the other hand, Figure 1D delineates the detection of trans-cleavage activity facilitated by Cas12a, employing RPA products as the intended targets. The findings from agarose gel electrophoresis affirm that the RPA reaction endures even when diluted to copies of plasmid DNA. The Figure 1D vividly showcases a discernible signal that significantly exceeds the background level, signifying the identification of the tar-

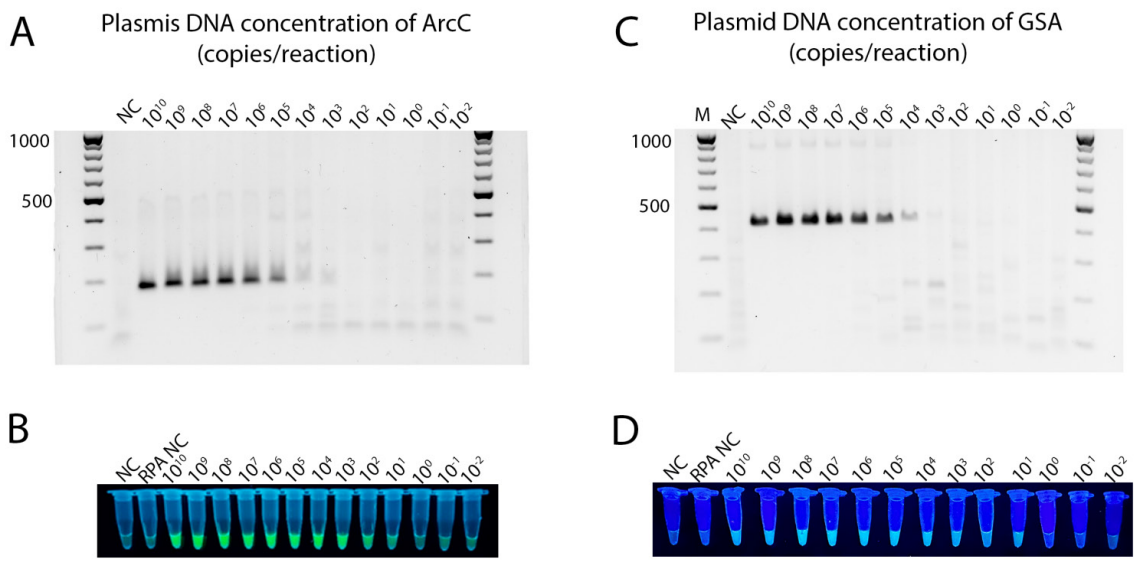

Figure 1. Evaluation of the analytical sensitivity of CRISPR/Cas using RPA technology for the detection of *S. aureus* ArcC and GSA genes. (A) An image of electrophoresis in an agarose gel of RPA products with a 10-fold sequentially diluted positive plasmid with the ArcC gene as a target; (B) The results of the trans-cleavage activity of the product. The image was obtained in ultraviolet light using a smartphone camera; (C) An image of electrophoresis in an agarose gel of RPA products with a 10-fold sequentially diluted positive plasmid with the GSA gene as a target; (D) Results of the trans-cleavage activity of the product. The image was obtained in ultraviolet light using a smartphone camera; RPA NC– negative control, RPA reaction without matrix DNA. NC - negative control of Cas12a trans-cleavage activity without a pre-amplification product.

geted genetic sequence. The established limit of detection, measured at copies per reaction, strongly indicates that the RPA-Cas12a method possesses a heightened sensitivity for detecting the *gsa* gene locus.

DISCUSSION

The successful integration of CRISPR/Cas12a-based analysis for *S. aureus* detection in plasmids with a positive control underscores its potential as a reliable and highly sensitive tool in the field of molecular diagnostics. The RPA-CRISPR/Cas12a analysis demonstrated excellent results for the GSA and ArcC genes. The detection limits for ArcC were 10^3 when using agarose gel analysis and 10^2 copies for fluorescence analysis, while for GSA, they were 10^4 with agarose gel analysis and 10^1 copies with fluorescent analysis. The combination of RPA and CRISPR/Cas12a methods provides a powerful and accessible platform for detecting *S. aureus* in samples, which holds significant value for diagnosing and monitoring various diseases.

In our study, we presented the results of research demonstrating the potential application of the CRISPR/Cas system in the field of molecular diagnostics, specifically for the detection of *S. aureus* and other bacterial pathogens. Our findings confirm that CRISPR/Cas represents a powerful tool for detecting specific genetic sequences, including those associated with this bacterial pathogen. One of the key aspects of our research was to develop a diagnostic method based on the CRISPR/Cas system for the detection of *S. aureus*. This process begins with the identification of unique genetic sequences inherent to this bacterial species. We utilized crRNAs targeted at amplified products from the bacterial genome isolated from samples. Once these sequences were identified, the CRISPR/Cas system could be effectively employed for the precise detection of *S. aureus*. It operates by using crRNAs to specifically target the genetic sequence of *S. aureus*, after which the Cas12a enzyme cleaves it, producing a fluorescent signal that is easily detectable.

Comparing our methodology to traditional diagnostic methods, such as microbiology and PCR diagnostics, we observed several advantages. Our method is faster, more sensitive, and specific, allowing for earlier detection of *S. aureus*. We also demonstrated high analytical sensitivity.

Additionally, we used the RPA method as a pre-amplification step for Cas12a-based diagnostics. This approach proved to be rapid, straightforward, and highly sensitive, with the potential to enhance the accuracy of nucleic acid detection.

It is important to note that our study represents an initial step in the development of this technology. Despite its promise, we recognize the importance of further research and comparisons with traditional diagnostic methods. Conducting comparative analyses with PCR and microbiology methods will provide a more comprehensive evaluation of the effectiveness and potential of our approach.

Overall, our research highlights the potential revolution in molecular diagnostics facilitated by the CRISPR/Cas technology. This combination of RPA and CRISPR/Cas12a could become a powerful tool for detecting various diseases and pathogens.

CONCLUSION

In conclusion, this study unveils a promising avenue in the realm of molecular diagnostics through the application of the RPA-CRISPR/Cas12a method. The diagnostic approach based on RPA-CRISPR/Cas12a exhibits notable advantages over traditional methods, such as PCR and microbiology. Its practical simplicity facilitates result acquisition, offering a valuable asset for a broad spectrum of clinical and research applications.

This research marks an initial step in the development of this technology. Future investigations should focus on comparative analyses with existing diagnostic methods, including PCR and microbiology, to further validate and optimize the protocol's effectiveness. Such endeavors will provide a comprehensive assessment of the potential and prospects of our approach in diverse clinical and research scenarios.

In essence, the study signifies a promising shift in molecular diagnostics, where the RPA-CRISPR/Cas12a combination emerges as a powerful and versatile tool for the detection of various diseases and pathogens. Its attributes of speed, accessibility, and enhanced sensitivity position it as a significant contributor to the ongoing efforts in disease management and prevention.

FUNDING

This research was funded by the Ministry of Agriculture of the Republic of Kazakhstan (BR10764944) and the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP09259771).

ACKNOWLEDGMENTS

We thank Dr. G. Chuzhebayeva (associate professor, Department of Veterinary Sanitation, Kostanay Regional University named after A. Baitursynov) for providing genomic DNA of *S. aureus* isolates.

LITERATURE

1. Ahmad-Mansour N., Loubet P., Pouget C., Dunyach-Remy C., Sotto A., Lavigne J. P., Molle V. Staphylococcus aureus Toxins: An Update on Their Pathogenic Properties and Potential Treatments // Toxins (Basel). – 2021. – V. 13, № 10.

2. Lee A. S., de Lencastre H., Garau J., Kluytmans J., Malhotra-Kumar S., Peschel A., Harbarth S. Methicillin-resistant Staphylococcus aureus // Nat Rev Dis Primers. – 2018. – V. 4. – P. 18033.

3. Cheung G. Y. C., Bae J. S., Otto M. Pathogenicity and virulence of Staphylococcus aureus // Virulence. – 2021. – V. 12, № 1. – P. 547-569.

4. Oliveira D., Borges A., Simões M. Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases // Toxins (Basel). – 2018. – V. 10, № 6.

5. Wu S., Duan N., Gu H., Hao L., Ye H., Gong W., Wang Z. A Review of the Methods for Detection of Staphylococcus aureus Enterotoxins // Toxins (Basel). – 2016. – V. 8, № 7.

6. Hirvonen J. J. The use of molecular methods for the detection and identification of methicillin-resistant Staphylococcus aureus // Biomark Med. – 2014. – V. 8, № 9. – P. 1115-25.

7. Kaminski M. M., Abudayyeh O. O., Gootenberg J. S., Zhang F., Collins J. J. CRISPR-based diagnostics // Nat Biomed Eng. – 2021. – V. 5, № 7. – P. 643-656.

8. Amer H. M., Abd El Wahed A., Shalaby M. A., Almajhdi F. N., Hufert F. T., Weidmann M. A new approach for diagnosis of bovine coronavirus using a reverse transcription recombinase polymerase amplification assay // J Virol Methods. – 2013. – V. 193, № 2. – P. 337-40.

9. Koonin E. V., Makarova K. S., Zhang F. Diversity, classification and evolution of CRISPR-Cas systems // Curr Opin Microbiol. – 2017. – V. 37. – P. 67-78.

10. Zetsche B., Gootenberg J. S., Abudayyeh O. O., Slaymaker I. M., Makarova K. S., Essletzbichler P., Volz S. E., Joung J., van der Oost J., Regev A., Koonin E. V., Zhang F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system // Cell. – 2015. – V. 163, № 3. – P. 759-71.

REFERENCES

1. Ahmad-Mansour N., Loubet P., Pouget C., Dunyach-Remy C., Sotto A., Lavigne J. P., Molle V. Staphylococcus aureus Toxins: An Update on Their Pathogenic Properties and Potential Treatments // Toxins (Basel). – 2021. – V. 13, № 10.

2. Lee A. S., de Lencastre H., Garau J., Kluytmans J., Malhotra-Kumar S., Peschel A., Harbarth S. Methicillin-resistant Staphylococcus aureus // Nat Rev Dis Primers. – 2018. – V. 4. – P. 18033.

3. Cheung G. Y. C., Bae J. S., Otto M. Pathogenicity and virulence of Staphylococcus aureus // Virulence. – 2021. – V. 12, № 1. – P. 547-569.

4. Oliveira D., Borges A., Simões M. Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases // Toxins (Basel). – 2018. – V. 10, № 6.

5. Wu S., Duan N., Gu H., Hao L., Ye H., Gong W., Wang Z. A Review of the Methods for Detection of Staphylococcus aureus Enterotoxins // Toxins (Basel). – 2016. – V. 8, № 7.

6. Hirvonen J. J. The use of molecular methods for the detection and identification of methicillin-resistant Staphylococcus aureus // Biomark Med. – 2014. – V. 8, № 9. – P. 1115-25.

7. Kaminski M. M., Abudayyeh O. O., Gootenberg J. S., Zhang F., Collins J. J. CRISPR-based diagnostics // Nat Biomed Eng. – 2021. – V. 5, № 7. – P. 643-656.

8. Amer H. M., Abd El Wahed A., Shalaby M. A., Almajhdi F. N., Hufert F. T., Weidmann M. A new approach for diagnosis of bovine coronavirus using a reverse transcription recombinase polymerase amplification assay // J Virol Methods. – 2013. – V. 193, № 2. – P. 337-40.

9. Koonin E. V., Makarova K. S., Zhang F. Diversity, classification and evolution of CRISPR-Cas systems // Curr Opin Microbiol. – 2017. – V. 37. – P. 67-78.

10. Zetsche B., Gootenberg J. S., Abudayyeh O. O., Slaymaker I. M., Makarova K. S., Essletzbichler P., Volz S. E., Joung J., van der Oost J., Regev A., Koonin E. V., Zhang F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system // Cell. – 2015. – V. 163, № 3. – P. 759-71.

РАЗВИТИЕ МЕТОДОВ ВЫЯВЛЕНИЯ *STAPHYLOCOCCUS AUREUS* С ИСПОЛЬЗОВАНИЕМ ТЕХНОЛОГИИ ФЛУОРЕСЦЕНТНОГО АНАЛИЗА RPA-CRISPR-CAS12A

Зейнулин М.[1], Аманжолова М.[2], Шайзадинова А.[3], Абельденов С.[1]

[1] Казахский агротехнический Университет имени С. Сейфуллина, Астана, Казахстан
[2] Национальный центр биотехнологии, Астана, Казахстан 010000
[3] Казахский национальный университет им. Аль-Фараби, Алматы, Казахстан.

АННОТАЦИЯ

В этом исследовании исследуется преимущества диагностики на основе CRISPR/Cas для точной идентификации *Staphylococcus aureus* (*S. aureus*), особенно в сочетании с рекомбиназной полимеразной амплификацией (RPA). *S. aureus*, грамположительная бактерия, представляет значительные проблемы из-за своей устойчивости к антибиотикам и способности вызывать различные инфекции. Хотя существующие методы диагностики обеспечивают точность, они часто включают в себя трудоемкие процедуры и требуют специализированного оборудования. Наше исследование представляет перспективный подход, который использует специфичность технологии CRISPR/Cas для нацеливания на генетические последовательности, позволяя точно обнаруживать *S. aureus*. Благодаря комбинации CRISPR/Cas и RPA мы ускоряем процесс обнаружения, обеспечивая быструю и высокочувствительную идентификацию патогена. Примечательно, что наше исследование демонстрирует отличные результаты для генов *gsa* и *arcC*, подтверждая потенциал этого метода как надежного и высокочувствительного инструмента молекулярной диагностики. В сравнении с традиционными методами диагностики, такими как микробиология и ПЦР, наш подход дает преимущества с точки зрения скорости и чувствительности. Кроме того, включение RPA в качестве этапа предварительной амплификации повышает точность определения нуклеиновых кислот. Хотя наше исследование представляет собой начальный шаг в технологическом прогрессе, оно подчеркивает потенциал диагностики на основе CRISPR/Cas для революционного выявления заболеваний и их лечения. RPA-CRISPR/Cas обладает потенциалом для выявления широкого спектра заболеваний и патогенов с широким применением, как в медицинской, так и в научной областях.

Ключевые слова: *Staphylococcus aureus*, диагностика, CRISPR/Cas, RPA, Cas12a, GSA *ген,* ArcC *ген.*

RPA-CRISPR-CAS12A ФЛУОРЕСЦЕНТТІ ТАЛДАУ ТЕХНОЛОГИЯСЫН ҚОЛДАНА ОТЫРЫП, *STAPHYLOCOCCUS AUREUS* АНЫҚТАУДЫҢ АРТЫҚШЫЛЫҚТАРЫ

Зейнулин М.[1], Аманжолова М.[2], Шайзадинова А.[3], Абельденов С.[1]

[1] С. Сейфуллин атындағы қазақ агротехникалық университеті, Қазақстан, Астана
[2] Ұлттық биотехнология орталығы, Қазақстан, Астана, 010000
[3] Әл-Фараби атындағы ҚазҰУ, Алматы, Қазақстан.

ТҮЙІН

Бұл зерттеу *Staphylococcus aureus (S. aureus)* дәл анықтау үшін CRISPR/CAS негізіндегі диагностиканың артықшылықтарын зерттейді, әсіресе рекомбиназа-полимеразды күшейтумен (RPA) біріктірілген. *S. aureus*, әмбебап грам-позитивті бактерия, антибиотиктерге төзімділігі мен әртүрлі инфекцияларды тудыру қабілетіне байланысты айтарлықтай қиындықтар туғызады. Қолданыстағы диагностикалық әдістер дәлдікті қамтамасыз еткенімен, олар көбінесе көп уақытты қажет ететін процедураларды қамтиды және арнайы жабдықты қажет етеді. Біздің зерттеуіміз *S. aureus*-ті дәл анықтауға мүмкіндік беретін генетикалық тізбектерді нысанаға алу үшін CRISPR/Cas технологиясының ерекшелігін пайдаланатын перспективалық тәсілді ұсынады. CRISPR/Cas және RPA синергетикалық комбинациясы арқылы біз патогеннің генетикалық материалын жылдам және жоғары сезімтал сәйкестендіруді қамтамасыз ету арқылы анықтау процесін жылдамдатамыз. Бір қызығы, біздің зерттеуіміз *gsa* және *arcC* гендері үшін тамаша нәтижелерді көрсетеді, бұл әдістің сенімді және жоғары сезімтал Молекулалық диагностика құралы ретіндегі әлеуетін растайды. Микробиология және ПТР сияқты дәстүрлі диагностикалық әдістермен салыстырғанда, біздің көзқарасымыз жылдамдық пен сезімталдық тұрғысынан артықшылықтар береді. Сонымен қатар, RPA-ны алдын-ала күшейту қадамы ретінде қосу нуклеин қышқылдарын анықтаудың дәлдігін арттырады. Біздің зерттеуіміз технологиялық прогрестің бастапқы қадамы болғанымен, ол ауруларды революциялық анықтау және емдеу үшін CRISPR/Cas негізіндегі диагностиканың әлеуетін көрсетеді. RPA-CRISPR/Cas медициналық және ғылыми салаларда кеңінен қолданылатын аурулар мен патогендердің кең ауқымын анықтау мүмкіндігіне ие.

Түйін сөздер: *Staphylococcus aureus*, диагностика, CRISPR/Cas, RPA, Cas12a, GSA ген, Arc ген.