Harnessing bioinformatics to identify unique gene sequences tailored for the specific detection of *Staphylococcus aureus* by real-time PCR

Le To Lan, Dang Diem Quynh, Dang Phuong Nam, Dang Phuong Thuy, Duong Thi Thanh Loan, Nguyen Thi Xuan, Nguyen Thi Nhung*

Institute of Materials, Biology and Environment/Academy of Military Science and Technology, 17 Hoang Sam, Nghia Do, Hanoi, Vietnam.
*Corresponding author: tepnhung@gmail.com
Received 27 Oct. 2025; Revised 5 Dec. 2025; Accepted 26 Dec. 2025; Published 25 Feb. 2026.
DOI: https://doi.org/10.54939/1859-1043.j.mst.109.2026.95-103

ABSTRACT

Staphylococcus aureus is a major pathogen responsible for a wide range of foodborne illnesses. When the density of S. aureus reaches 10^5 CFU/mL, the pathogen begins producing enterotoxin - the main agent responsible for illness. While past studies mainly targeted enterotoxins or antibiotic-resistant strains, recent works show that real-time PCR enables sensitive detection at low bacterial levels, before toxin production. This study used bioinformatics tools to evaluate seven candidate genes (mecA, mecC, femA, nuc, sa442, coa, and spA) and identify the most specific qPCR target. Sequence conservation and specificity were analyzed with Geneious and NCBI BLAST, revealing the nuc gene (positions 430–566) as the most reliable marker. Experimental qPCR using designed primers and probes demonstrated the highest specificity and optimal performance for the nuc gene. The results also confirmed that although sa442 is highly specific, it does not detect all S. aureus strains, thereby supporting the accuracy of the bioinformatic approach. These highlight the value of bioinformatics in selecting exact materials for qPCR kit development for diagnostics in food safety control.

Keywords: Bioinformatics; Real-time PCR; *S. aureus*; Nuc.

1. INTRODUCTION

Staphylococcus aureus (S. aureus) is a Gram-positive bacterium and has been a significant threat to public health and food safety due to its ability to produce enterotoxins and multi-drug resistance. Global outbreaks have been reported many times and in Vietnam, they often occurs in communal kitchens or small-scale facilities with poor hygiene, with notable cases in Ho Chi Minh City (2010, 2018), Ninh Binh (2018), and Ha Giang, Vinh Phuc (2017, 2018) [1]. According to the U.S. FDA, *S. aureus* is able to pathogenic when bacterial density reaches 10^6 CFU/mL and begins producing toxins at 10^5 CFU/mL [2]. Therefore, a rapid and accurate method to detect *S. aureus* is a top priority in food safety control. The most suitable method is real-time PCR (qPCR) targeting species-specific genes.

S. aureus causes severe infections like pneumonia, bloodstream and skin infections. Its genome contains ~ 2,700 genes on a circular chromosome and extra- chromosomal elements (plasmids, phages, mobile genetic elements (MGEs)), enabling adaptability via horizontal gene transfer for resistance and virulence. The core genome governs metabolism, cell cycle, and virulence, including toxin production, invasion, and immune evasion [3]. Key genes include *nuc* gene, which play a role in immune invasion and widely used for *S. aureus* detection [4]; *femA* gene, involved in peptidoglycan biosynthesis and *mecA* expression for MRSA [5]; *sa442* - a conserved sequence [6] and *coa* and *spA*, which promote immune evasion and infection spread [7]. The accessory genome on MGEs like SaPIs, and SCCmec carries *mecA* (MRSA-specific, transferable) and *mecC*, ~ 69% identical to *mecA*, encodes PBP2a.

Through natural evolution and horizontal gene transfer, *S. aureus* acquires resistance and virulent genes, which enhances its antibiotic resistance and pathogenicity. These traits are

governed by specific genes, making the selection of an appropriate target gene crucial for accurate *S. aureus* detection by qPCR. Medical device companies worldwide have developed proprietary commercial kits targeting different specific genes (e.g., BD: SCCmec-orfX, *mecA*, *mecC*, *nuc*; Roche: SCCmec-orfX, *mecA*; Cepheid: SCCmec, *mecA*, *spA*; Kogene: *femA*) [8-11]. Even though, the *SCCmec-orfX* junction, commonly used for MRSA detection, it was not detected in 14 novel MRSA strains with variant sequences and single nucleotide polymorphisms (SNPs) [9]. MRSA strains with the *mecC* gene can be misidentified as methicillin-sensitive *S. aureus* (MSSA) due to diagnostic challenges. Although related to methicillin resistance or other pathogenic mechanisms, target genes have not been fully evaluated for coverage and specificity, making it difficult to choose a single reliable gene for detecting *S. aureus*.

The emergence of bioinformatics as a powerful tool in molecular biotechnology, combined with the rapid expansion of genomic databases, has greatly enhanced the ability to analyze and select specific target genes for the detection of pathogenic organisms. This study aims to utilize bioinformatics to identify unique gene sequences and optimal target regions in *S. aureus*, enabling the design of improved primer–probe set for qPCR. The bioinformatic-based gene selection was experimentally validated, confirming the effectiveness of bioinformatic tools in identifying specific targets for *S. aureus* detection. The ultimate goal is to prepare a robust material for developing a sensitive, specific, and rapid real-time PCR kit for the detection of *S. aureus* in food samples.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Instrumentation

Real-time PCR MX 3005P System (Agilent Technology, USA), Bio-Rad PCR (USA), electrophoresis (Cleaver Scientific, UK), Biocen 22 R centrifuge (Orto alresa, Spain).

Software and databases: ApE software, Geneious Prime (2024.0) (New Zealand), NCBI GenBank, NCBI BLAST and Primer-BLAST, IDT PrimerQuest Tool, Neb Calculator, Oligo IDT.

2.1.2. Experimental materials

DNA extracts of *S. aureus* ATCC 25923, *S. aureus* 03, *S. aureus* 93587, *S. aureus* 9358, *S. aureus* 57, *S. aureus* 43 were from the Military Institute of Preventive Medicine.

Chemicals: Taq Pro Multiple Probe qPCR Mix (Vazyme, Taiwan), Primers and probes (IDT, USA), 100 bp DNA Ladder (Thermo Fisher Scientific, USA).

2.2. Methods

2.2.1. Target gene selection

Target gene candidates were compiled based on a comprehensive review of previously published scientific literature and those commonly used by commercial kits to detect *S. aureus*, including *mecA, mecC, femA, nuc, sa442, coa*, and *spA*. The full-length sequences of these genes were retrieved from the NCBI GenBank database, from strains *S. aureus* ATCC 25923 (NZ_CP009361.1) and used as queries in BLAST analysis to assess species specificity.

2.2.2. Specific region selection

The selected genes sequences were aligned using multiple sequence alignment (MSA) from Geneious to identify highly conserved regions for primer and probe design.

2.2.3. Primers and probe design

Primers and probes were designed using NCBI Primer-BLAST and IDT PrimerQuest Tool, with secondary structures (hairpins, self-dimers, and hetro-dimers) evaluated via IDT OligoAnalyzer. Primers with unfavorable structures (Gibbs free energy $\Delta G < -9$ kcal/mol or hairpins at 3' end) were excluded. TaqMan probes were ~50 nucleotides (nt) downstream of the

forward primer and designed to meet standard criteria. Melting and annealing temperatures (T_m and T_a) were calculated using the NEB Tm Calculator, ensuring the probe's T_m was 5 - 10 °C higher than that of the primers and free of significant secondary structures. All sequences were checked for specificity using BLAST and validated by Geneious to avoid cross-reactivity with non-*S. aureus* DNA.

2.2.4. In-silico evaluation

In-silico PCRs were conducted using Primer-BLAST to simulate the amplification in order to evaluate the designed primer/probe set. The simulation provides a means to confirm specific amplification of the intended target region, with the correct product size and no non-target binding.

2.2.5. Real-time PCR assay validation

qPCR (20 µL) was performed with 10 µL 2x qPCR Mix, primer at 50 nM, probe at 25 nM, total DNA template at 10 ng. The negative control was run with mili-Q water instead of DNA template. The thermal cycle protocol started at 37 °C for 2 minutes, initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 10 seconds, annealing step at 60 °C for 30 seconds.

3. RESULTS AND DISCUSSION

3.1. Target gene collection

Seven candidate gene sequences (mecA, mecC, femA, nuc, sa442, coa, and spA) commonly reported as molecular markers for the detection of *S. aureus* were retrieved from *S. aureus* strain ATCC 25923.

Table 1. *Information on target genes.*

Gene	No. of *S. aureus* hits/Total hits	% *S. aureus* hits	% identical sites in *S. aureus*	Presented in other species and identity (%)	Note
mecA	2906/3137	92.63%	97.2 – 100%	• *S. schweitzeri* (99.7%) • *S. taiwanensis (*80,4%) • *S. shleiferi* (88.1 – 88.4%)	
mecC	21/57	36.84%	92 – 100%	• *S. caprae (*99.9%) • *S. sciuri* (96 – 96.1%) • *Mammaliicocus lentus* (94%) • *S. stepanoricii* (98.7 – 99.2%)	
femA	2990/3163	94.53%	85.5 – 100%	• *S. argenteus* (95.5 – 95.8%) • *S. milleri* (94.5%)	
nuc	3081/3166	97.31%	82.1 – 100%	• *S. argenteus* (81.7 – 82.8%)	candidate target gene
sa442	4979/5000	99.58%	98.4 – 100%	• *S. schweitzeri* (89.5%)	candidate target gene
coa	4972/5000	99.44	74.5 – 100%	• *Bacillus subtilis* (96.6%) • *Klebsiella pneumonia* (99.8%) • *Enterobacter cloacae* (100%)	
spA	4907/5000	98.14%	84.7 – 100%	• *S. schweiteri (86.2%)* • *S. argenteus* (88.6 – 100%)	

mecA and *femA* are primarily associated with resistant in MRSA and the BLAST results showed that they are not consistently present in all *S. aureus* strains (92.63% and 94.53%, respectively) (Table 1). Besides, they are spontaneously present in other *Staphylococcus*: *S. schweitzeri*, *S. taiwanensis*, and *S. shleiferi* for *mecA*; *S. argenteus* and *S. milleri* for *femA* (88.1–99.7%), leading to cross-reactivity and reduced specificity. Notably, *mecC* is present in only 36.84% of strains, reducing the accuracy of detection. Although *coa* and *spA* are present in 99.44% and 98.14%, respectively, they also show high identity with other species – *B. subtilis* (96.6%), *K. pneumoniae* (99.8%), *E. cloacae* (100%), and have limited intraspecies coverage (74.5% and 84.7%), making them unsuitable for specific qPCR. The above results emphasize the importance of BLAST over *in-silico* PCR for *S. aureus* detection, since *in-silico* PCR suggested that the *mecA* and *coa* genes were suitable qPCR targets for *S. aureus* [12], whereas this study demonstrated otherwise.

For *nuc* and *sa442*, the BLAST results showed that they share the highest genetic stability and minimal cross-reactivity with non-*S. aureus* species. Both genes display lower homology to other species: *nuc* is only homologous to *S. argenteus* (81.7–82.8%), while *sa442* shared 89.5% similarity with sole *S. schweitzeri*. Notably, *nuc* is not a mobile genetic element and is therefore not subject to horizontal gene transfer like resistance genes such as *mecA* and *mecC.* Therefore, *nuc* and *sa442* were primarily selected as the most suitable molecular markers, with further analysis of conserved regions.

3.2. Determination of conserved and specific region

3.2.1. Conserved and specific region of nuc gene

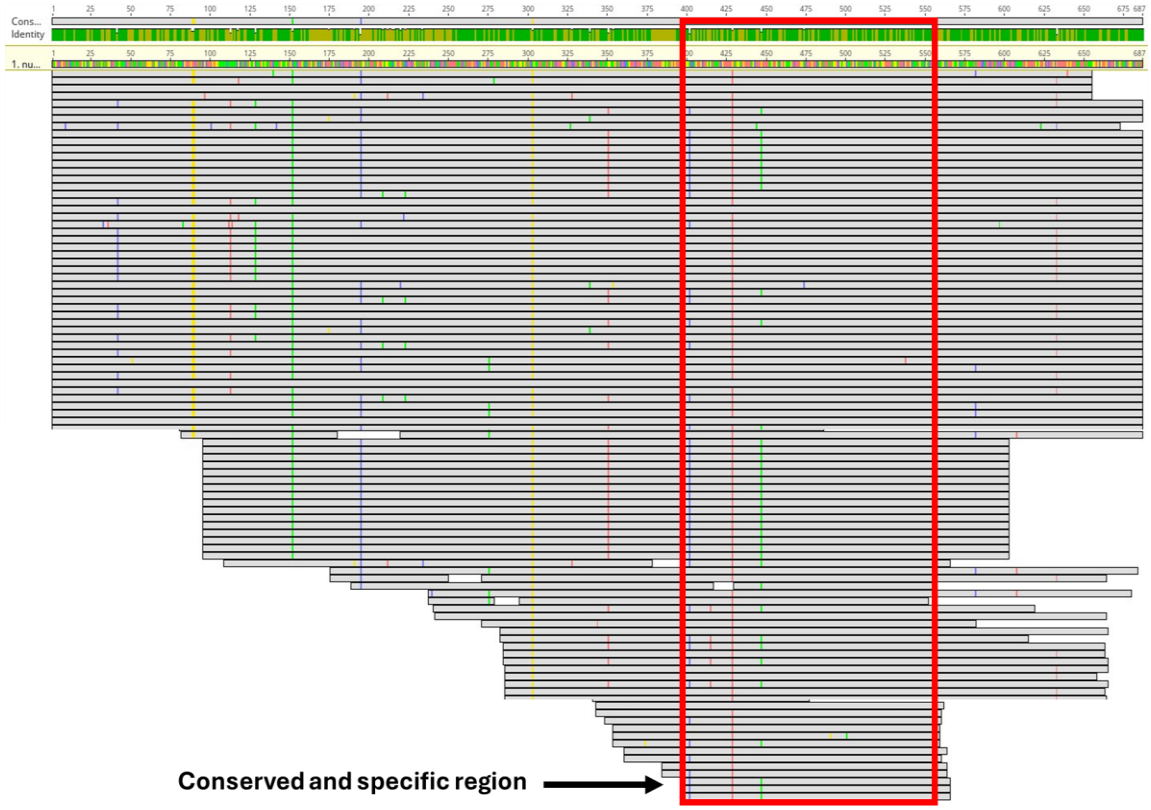

Figure 1. *Multiple sequence alignment (MSA) of the nuc gene among different S. aureus strains, generated using Geneious. The colored points (red - A, green – T, yellow – G, and purple – C) were SNPs. Red boxed region: highly conserved and specific region.*

To select a suitable region for target, the sequence must show high genetic stability with minimal mutations or SNPs in homologous sequences. A MSA of 3,081 *S. aureus nuc* gene showed that region 430–566 possesses the highest stability and covers the shortest homologous sequence (Fig. 1). This region is consistently present in all reported *S. aureus* homologous sequences with only a few SNPs in some strains, which did not significantly affect the stability of this region, confirming its specificity. This region was re-examined using BLAST against *S. argenteus*, revealing multiple mutations within the primer-binding sites and other sites, thereby ensuring the absence of non-specific amplification (data not shown).

3.2.2. Conserved and specific region of sa442 gene

Figure 2. *Multiple sequence alignment (MSA) of the sa442 gene among different S. aureus strains, generated using Geneious. The left sequence corresponds to 256 nucleotides region, the right sequence to 190 nucleotides region. The colored points (red-A, green-T, yellow-G, and purple-C) were SNPs.*

The *sa442* gene, 442 nt in length, comprises 256 nt homologous to the glutamate synthase gene and 190 nt corresponding to the phosphomevalonate kinase gene [6]. MSA revealed that the 256-nt region is present in a subset of *S. aureus* strains but absent in those covered by the 190-nt region (Fig. 2). Therefore, neither the 256-nt nor the 190-nt region was selected as the primary target. Nonetheless, in this study, primers and a probe were still designed for the 256-nt region to experimentally verify the predictions from the bioinformatic analysis.

3.3. Primer and probe design

The primer and probe designed for *nuc* and *sa442* were validated for specificity using BLAST and cross-checked in Geneious to ensure no significant homology with non-*S. aureus* sequences (data not shown). All primer pairs met the required design criteria (table 2), confirming their potential for effective amplification across *S. aureus* while minimizing cross-reactivity.

Table 2. *List of primers and probes designed for qPCR.*

Primer/ probe	Sequence 5'-3'	Product size (bp)	T_m (°C)	Hairpin ΔG (kcal/mole)	Self-dimer ΔG (kcal/mole)	Hetero-dimer ΔG (kcal/mole)
nuc-qPCR-Fw	ATTGAAGTCGAGTTTGACAAAGG	111	54	-2.62	-6.61	-6.61 with *nuc-Rv*
nuc-qPCR-Rv	CAAGCCTTGACGAACTAAAGC		55	-0.33	-5.5	-9.75 with *nuc*-probe
nuc-probe-FAM	56/FAM/ACGTGGCTT/ZEN/AGCGTA TATTTATCCTGATGGA/3IABkFQ		62	-3.05	-6.3	-3.89 with *nuc*-Fw
sa422-qPCR-Fw	CACGACTAAATAAACGCTCATTCG	156	55	-0.63	-5.19	-5.19 with *sa442-Rv*
sa422-qPCR-Rv	GTATGACCAGCTTCGGTACTAC		56	-1.30	-6.34	-5.00 with sa442-probe
sa422-probe-FAM	FAM-CTCATTACGTTGCATCGGA AACATTGTGTTCTGT-BHQ13'		63	-2.16	-7.05	-6.90 with *sa442*-Fw

3.4. *In-silico* evaluation

In-silico PCR analysis confirmed that the designed primer set successfully amplified the target genes (111 bp for *nuc*; 156 bp for *sa442*) of the bacterial strain *S. aureus* TPS3156 (AP023034.1) (Fig 3 and 4). Both primer pairs exhibited low 3′ complementarity, suggesting a low risk of primer–dimer formation. However, moderate self-complementarity (score = 5.00-7.00) could potentially affect amplification efficiency and should be considered to optimize performance in real experiments.

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATTGAAGTCGAGTTTGACAAAGG	23	57.58	39.13	7.00	3.00
Reverse primer	CAAGCCTTGACGAACTAAAGC	21	57.81	47.62	7.00	2.00

Products on target templates

>AP023034.1 Staphylococcus aureus TPS3156 DNA, complete genome

```
product length = 111
Forward primer  1       ATTGAAGTCGAGTTTGACAAAGG  23
Template        861499  ......................  861521

Reverse primer  1       CAAGCCTTGACGAACTAAAGC  21
Template        861609  .....................  861589
```

Figure 3. *In-silico PCR results of nuc gene using Primer-BLAST.*

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CACGACTAAATAAACGCTCATTCG	Plus	24	1146	1169	58.82	41.67	6.00	2.00
Reverse primer	GTATGACCAGCTTCGGTACTAC	Minus	22	1301	1280	58.04	50.00	5.00	5.00
Product length	156								

Products on potentially unintended templates

>AP023034.1 Staphylococcus aureus TPS3156 DNA, complete genome

```
product length = 156
Forward primer  1        CACGACTAAATAAACGCTCATTCG  24
Template        2570081  ........................  2570104

Reverse primer  1        GTATGACCAGCTTCGGTACTAC  22
Template        2570236  ......................  2570215
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Figure 4. *In-silico PCR results of sa442 gene using Primer-BLAST.*

3.5. qPCR validation

qPCR assays were conducted with 1 standard and 5 isolated *S. aureus* strains to assess the amplification of the *nuc* and *sa442* gene. Using the designed probes and primers, the assays successfully amplified the target genes with high amplification. For the *nuc*, all five *S. aureus* strains were successfully detected, exhibiting consistent Ct values and stable fluorescence signals (Fig 6A). In contrast, amplification of the *sa442* gene was observed in just four strains, with variable Ct values and inconsistent fluorescence. Strain *S. aureus* 9357 showed no amplification signal with *sa442* (Fig 6B), indicating that this strain carries the *nuc* but lacks *sa442* gene. This result is consistent with Klaassen *et al*., 2003 [13] who reported that *sa442* was present in fewer strains compared to *nuc*. These experimental findings suggest that the *sa442* gene exhibits lower sensitivity and a narrower strain coverage compared to the *nuc* gene. In addition, the specificity of the designed *nuc* gene primers was rigorously tested against a panel of non-*S. aureus* bacterial strains such as: *Shigella*, *Salmonella*, *E. coli, P. aeruginosa, etc.* (data not shown). The absence of detectable signals in these non-target species clearly demonstrated that no cross-amplification occurred. Therefore, the experimental results corroborate the predictions by bioinformatic tools, proving an accurate tool for early experimental outcome prediction, reducing experimental costs while still providing valuable scientific insights. Consequently, the *nuc* gene was selected as the molecular marker for *S. aureus* detection by qPCR.

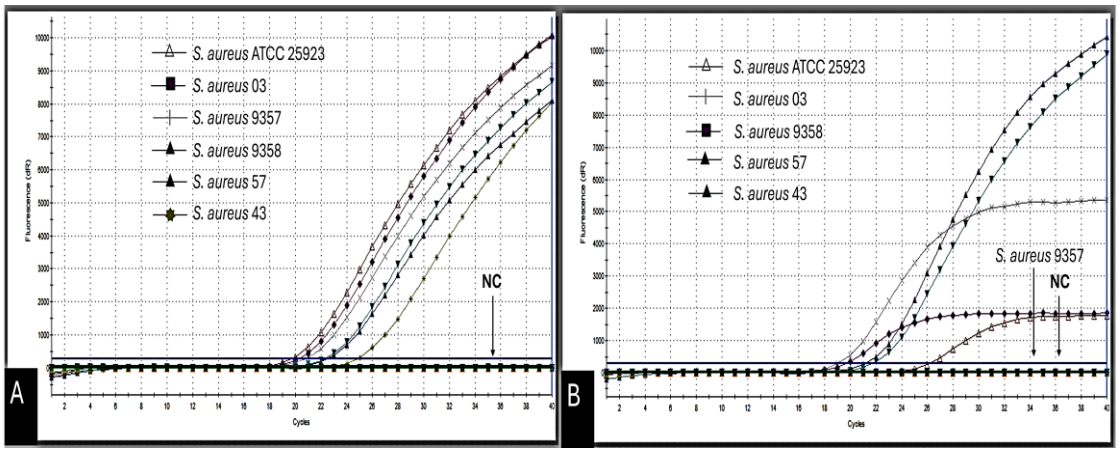

Figure 5. *qPCR assay for S. aureus detection. (A) using nuc gene to detect S. aureus; (B) using sa442 gene to detect S. aureus; NC: Negative control.*

4. CONCLUSIONS

This study demonstrates the value of bioinformatics in developing accurate diagnostics for *S. aureus*. By leveraging bioinformatic software, tools and *in silico* analyses, we identified a highly specific region of the *nuc* gene, making it a reliable target for molecular detection. Using online tools, specific primers and probes were designed and confirmed their specificity before wet-lab testing. This approach minimized experimental workload, enhanced assay performance, and ensured broad strain coverage. The resulting qPCR assay confirmed a highly sensitive and specific detection of *S. aureus* and supported its potential for development of a detection kit. In conclusion, bioinformatics has proven to be powerful in the development of next-generation molecular diagnostic tools and applications.

Acknowledgement: *This study was carried out with financial support from the project titled: "Study on, design and fabrication of kits for simultaneous detection of three biological agents: Shigella, Staphylococcus aureus, and Salmonella spp. using multiplex real-time PCR technique" funded by Academy of Science and Technology, General Staff of the Vietnam People's Army.*

REFERENCES

[1]. Quyen D. V., Lanh P. T., Gahyun L., Hoa N. T., Phuoc M. H., *"Isolation and phylogenetic analysis of Staphylococcus aureus strains isolated from meat in traditional markets in Ha Noi"*, Academia Journal of Biology, vol. 47, no. 1, pp. 63–74, (2025). DOI: 10.15625/2615-9023/21635.

[2]. U.S. Food and Drug Administration*, "Staphylococcus aureus toxin formation in hydrated batter mixes,"* in Fish and Fishery Products Hazards and Controls Guidance, 4th ed., Washington, DC: FDA, ch. 15, pp. 291–298, (2022).

[3]. Młynarczyk A., Młynarczyk G., Jeljaszewicz J., *"The genome of Staphylococcus aureus: A review"*, Zentralblatt für Bakteriologie: International Journal of Medical Microbiology, vol. 287, no. 4, pp. 277–314, (1998). DOI: 10.1016/S0934-8840(98)80165-5.

[4]. Yang Y., Su X., Yuan Y., Kang C., Li Y., Zhang W., Zhong X., *"Detection of Staphylococcus aureus in dairy products by polymerase chain reaction assay"*, Agricultural Sciences in China, vol. 6, no. 7, pp. 857–862, (2007). DOI: 10.1016/S1671-2927(07)60122-9.

[5]. Kobayashi N., Wu H., Kojima K., Taniguchi K., Urasawa S., Uehara N., Kurokawa I., *"Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction"*, Epidemiology and Infection, vol. 113, no. 2, pp. 259–266, (1994). DOI: 10.1017/S0950268800051682.

[6]. Martineau F., Picard F. J., Roy P. H., Ouellette M., Bergeron M. G., *"Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus"*, Journal of Clinical Microbiology, vol. 36, no. 3, pp. 618–623, (1998). DOI: 10.1128/jcm.36.3.618-623.1998.

[7]. Khan A., Hussain R., Javed M. T., Mahmood F., *"Molecular analysis of virulent genes (coa and spa) of Staphylococcus aureus involved in natural cases of bovine mastitis"*, Pakistan Journal of Agricultural Sciences, vol. 50, no. 4, pp. 739–743, (2013).

[8]. Nielsen X. C., Madsen T. V., Engberg J., *"Evaluation of Xpert MRSA Gen 3 and BD MAX MRSA XT for meticillin-resistant Staphylococcus aureus screening in a routine diagnostic setting in a low-prevalence area"*, Journal of Medical Microbiology, vol. 66, no. 1, pp. 90–95, (2017). DOI: 10.1099/jmm.0.000411.

[9]. Yam W. C., Siu G. K. H., Ho P. L., Ng T. K., Que T. L., Yip K. T., Yuen K. Y., *"Evaluation of the LightCycler methicillin-resistant Staphylococcus aureus (MRSA) advanced test for detection of MRSA nasal colonization"*, Journal of Clinical Microbiology, vol. 51, no. 9, pp. 2869–2874, (2013). DOI: 10.1128/JCM.00488-13.

[10]. Ojha S. C., Chen K., Sun C., Ahmed S., Sheng Y.-J., Deng C.-L., *"Clinical relevance of Xpert MRSA/SA in guiding therapeutic decisions for Staphylococcal infections: A diagnostic test accuracy analysis"*, Infectious Diseases and Therapy, vol. 11, no. 3, pp. 1205–1227, (2022). DOI: 10.1007/s40121-022-00632-w.

[11]. *"Kogene catalogue.pdf – BiOBiZ"*, yumpu.com. (2025).

[12]. Suhaili Z., Johari S. A., Sajili M. H., Yahya A., Zakaria Z. A., Desa M. N. M., Ali A. M., *"In silico sPCR verification and simplex real-time PCR detection of methicillin-resistant Staphylococcus aureus (MRSA) from East Coast Malaysian clinical isolates"*, J. Infect. Public Health, vol. 9, no. 5, pp. 631–641, (2016).

[13]. Klaassen C. H. W., de Valk H. A., Horrevorts A. M., *"Clinical Staphylococcus aureus isolate negative for the sa442 fragment"*, Journal of Clinical Microbiology, vol. 41, no. 9, p. 4493, (2003). DOI: 10.1128/JCM.41.9.4493.2003.

TÓM TẮT

Ứng dụng tin sinh học trong xác định các trình tự gen đặc hiệu cho phát hiện *Staphylococcus aureus* bằng kỹ thuật real-time PCR

Staphylococcus aureus là một tác nhân gây bệnh quan trọng liên quan đến nhiều vụ ngộ độc thực phẩm. Khi mật độ vi khuẩn đạt 10^5 CFU/mL, S. aureus bắt đầu sản sinh enterotoxin - yếu tố chính gây bệnh. Trong khi các nghiên cứu trước đây chủ yếu tập trung vào enterotoxin hoặc các chủng kháng kháng sinh, các nghiên cứu gần đây cho thấy real-time PCR có thể phát hiện nhạy ở mật độ vi khuẩn thấp, trước khi độc tố được tạo ra. Nghiên cứu này sử dụng các công cụ tin sinh học để đánh giá 7 gen tiềm năng (mecA, mecC, femA, nuc, sa442, coa và spA) nhằm xác định gen mục tiêu đặc hiệu nhất. Độ bảo thủ và tính đặc hiệu của trình tự các gen được phân tích bằng Geneious và NCBI BLAST, cho thấy gen nuc đoạn 430–566 là đoạn gen đặc hiệu tin cậy nhất. Mồi và probe được thiết kế bằng công cụ tin sinh cho thấy độ đặc hiệu cao và hiệu suất qPCR tối ưu khi thí nghiệm thực tế, đồng thời xác nhận rằng mặc dù gen sa442 có tính đặc hiệu cao nhưng không xuất hiện ở tất cả các chủng S. aureus như gen nuc. Kết quả này nhấn mạnh giá trị của tin sinh học trong việc lựa chọn chính xác các vật liệu trước khi thực nghiệm để phát triển bộ kit qPCR phục vụ chẩn đoán kiểm soát an toàn thực phẩm.

Từ khoá: Tin sinh học; Real-time PCR; *S. aureus*; Nuc.