Original Article

Microbial Biosystems 10(2) (2025) 2025.355480
10.21608/mb.2025.355480.1240

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Study of elevation of *spdC* gene expression with increased antibiotic resistance and virulence genes of *Staphylococcus aureus* isolated from diarrheal infections

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ARTICLE INFO

Article history
Received 25 January 2025
Received revised 15 February 2025
Accepted 4 April 2025
Available online 1 June 2025

Corresponding Editors:
Atyaha, A.
Ahmed, L.

Keywords
Diarrheal patients,
genetic detection,
Mannitol salt agar,
RT-qPCR,
Vitek 2 system.

ABSTRACT

Numerous clinical infections are caused by the opportunistic pathogen *Staphylococcus aureus*, including gastrointestinal diseases such as diarrhea. Frequent resistance to antibiotics makes *S. aureus* infections a problem. The surface protein displaying C encoded by (*spdC*) gene is an important virulence factor, which plays a role in anchoring virulence factors and antibiotic resistance genes in the cell wall. The objective of the current study is to investigate the significance of the relationship between expression *spdC* gene, resistance to different antibiotics and virulence *S. aureus* isolates collected from diarrheal patients. Thirty clinical samples were obtained from diarrhea patients at Al-Diwaniyah Teaching Hospital, Women and Children Hospital in Al-Diwaniyah city/Iraq during the period from June to October 2024. *S. aureus* isolates were cultured and identified on blood agar, mannitol salt agar using biochemical tests specific for *S. aureus* isolates, as well as antibiotic susceptibility testing by Vitek 2 device, and genetic detection by RT-qPCR for 16S rRNA, *spdC, icaA* and *mecA* genes. Twelve *S. aureus* isolates were diagnosed at a rate of 40%. The isolates showed resistance to different types of antibiotics (I, II & III & IV) by Vitek 2. The expression (*icaA* 21.93, *spdC* 13.02 & *mecA* 5.03) genes was higher in the (IV) types, followed by types (III, II& I), respectively in expression. Strains of diarrheal *S. aureus* showed different resistance to multiple classes antibiotics. Expression analysis genes *spdC*, *icaA* & *mecA* were higher in isolates that showed multiple resistance to different types of antibiotics.

Published by Arab Society for Fungal Conservation

Introduction

Staphylococcus aureus is a pathogenic microorganism capable of causing illness in humans (Liana et al., 2015; Hmed et al., 2024; Mokabel et al., 2024; Jabbar & Abdul Wahid, 2025). It is recognized as a major contributor to zoonotic diseases and poses a significant threat to public health (Rahman et al., 2020). In addition, *S. aureus* is a well-known foodborne pathogen responsible for food poisoning outbreaks worldwide (Fetsch & Johler, 2018). It can cause bacteremia with considerable morbidity and mortality (Van Hal et al., 2012) and is a common agent of both hospital-acquired (nosocomial) and community-acquired infections (Uhlemann et al., 2014). The organism's capacity to cause severe infections is largely attributed to its diverse

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mechanisms of antibiotic resistance (Cheung et al., 2021; Mokabe et al., 2024).

Of particular concern are methicillin-resistant Staphylococcus aureus (MRSA) strains, which were initially identified in hospital settings but have increasingly been reported in community environments. These strains exhibit high virulence and infection rates (Lynch & Zhanel, 2023). Community-associated MRSA (CA-MRSA) strains combine methicillin resistance with significant pathogenic potential in ways not previously observed (Davis et al., 2004). A hallmark of *S. aureus* is the high expression of numerous virulence factors (Sila et al., 2009). Surface proteins play essential roles in adhesion, immune evasion, and biofilm formation.

S. aureus is an adept biofilm producer, enhancing its ability to persist, disseminate, and cause chronic infections (O'Neill et al., 2008). Its high genomic plasticity has led to extensive genetic variation, facilitating the emergence of highly resistant and virulent strains responsible for a wide array of diseases. Colonization, especially by methicillin-resistant strains, significantly contributes to its prevalence and associated mortality (Millan, 2016; Polívková et al., 2017; Haddad Kashani et al., 2018). The *mecA* gene encodes PBP2a, a penicillin-binding protein with reduced affinity for beta-lactam antibiotics, thereby conferring methicillin resistance (Mohammadzadeh et al., 2019).

Biofilms provide protective niches for bacterial cells, increasing their resistance to antibiotics. Biofilm-associated bacteria exhibit enhanced antimicrobial resistance and stronger adherence to host tissues (Sedarat & Taylor-Robinson, 2019). MRSA strains can express polysaccharide intercellular adhesin (PIA), synthesized through the transcription of the *icaA* operon, which contributes to biofilm formation (Jin et al., 2019).

The *spdC* gene, also known as *LyrA*, has recently been identified as a global virulence regulator in *S. aureus* (Poupel et al., 2018). It encodes a transmembrane protein containing an ABI domain, initially characterized in Lactococcus species due to its role in phage exclusion (Frankel et al., 2010). The *spdC* gene is positively regulated by the WalKR two-component system (TCS), which influences biofilm formation and virulence (Dubrac et al., 2008). *SpdC* modulates the activity of WalKR by interacting with the WalK histidine kinase and also regulates at least nine other *S. aureus* histidine kinases (Poupel et al., 2018). All sequenced *S. aureus* strains possess the *spdC* gene (Gründling et al., 2006).

The aim of this study is to investigate the clinical significance of *S. aureus* as a gastrointestinal pathogen, with a focus on its role in diarrheal infections. Additionally, the study seeks to explore the role of the *spdC* gene in the organism's virulence and pathogenicity, examine its association with other virulence genes, and assess the relationship between *spdC* expression and antibiotic resistance.

Materials and Methods

Collection and diagnosis of Staphylococcus aureus

In this study, thirty clinical samples were collected from patients of all ages presenting with diarrhea at Diwaniyah Teaching Hospital and the Women and Children Hospital in Diwaniyah City, Iraq, between June and October 2024. The samples were cultured on blood agar and mannitol salt agar and incubated at 37°C for 24 hours. *S. aureus* isolates were identified using standard microbiological techniques, including Gram staining, catalase test, coagulase test, oxidase test, and other biochemical assays (Procop et al., 2017).

Antimicrobial susceptibility testing

Antimicrobial resistance of the *S. aureus* isolates was determined using the VITEK 2 system with Gram-positive susceptibility cards, following the manufacturer's instructions (BioMérieux SA, France). The antibiotics included in the test panel were: Benzylpenicillin (P), Oxacillin (OX1), Gentamicin (GM), Ciprofloxacin (CIP), Moxifloxacin (MXF), Erythromycin (E), Clindamycin (CM), Linezolid (LNZ), Teicoplanin (TEC), Vancomycin (VA), Tetracycline (TE), Tigecycline (TGC), Fusidic Acid (FA), Rifampicin (RA), and Trimethoprim/Sulfamethoxazole (SXT), in accordance with CLSI guidelines (CLSI, 2024).

RT-qPCR primer design

Primers for real-time quantitative PCR (RT-qPCR) were designed using sequences obtained from NCBI GenBank and Primer3 Plus and synthesized by Scientific Researcher Co. Ltd. (Iraq). The primers used are shown in Table 1.

Real-time qPCR analysis

Relative gene expression levels of *spdC*, *mecA*, and *icaA* were quantified using RT-qPCR. The 16S rRNA gene was used as a housekeeping control. The following procedures were followed, as described by Poupel et al. (2018):

RNA extraction

Total RNA was extracted from *S. aureus* isolates using the Easy-BLUETM Total RNA Extraction Kit, according to the manufacturer's instructions.

DNase I treatment

Genomic DNA contamination was removed by incubating RNA samples with DNase I, buffer solution,

and DEPC-treated water at 37°C for 30 minutes. The enzyme was inactivated by adding 1 µL of stop solution and incubating the mixture at 65°C for 10 minutes.

Table 1 qPCR primers used in this study

Primer		Nitrogenous base sequence (5'-3')	Amplification size	NCBI Code / Reference
16S rRNA	F	GTGGAGGGTCATTGGAAACT	100 bp	Bakr et al. (2022)
	R	CACTGGTGT TCCTCCATATCTC		
SpdC	F	GCTTCAATGACATTTGGCCTTA	100 bp	Bakr et al. (2022)
	R	CTGCAACGATTGCTGTTGAAATG		
mecA	F	ACTGCTATCCACCCTCAAACAG	148 bp	KC243783.1
	R	TCTGGAACTTGTTGAGCAGAGG		
icaA	F	CGCTACGAGAAAAAGAATATGGC	93 bp	KU670830.1
	R	TGCGACAAGAACTACTGCTG		

cDNA synthesis

Complementary DNA (cDNA) was synthesized from DNase-treated RNA using random hexamer primers and the AccuPower® RocketScript™ RT PreMix kit. The mixture was briefly centrifuged and incubated at 42°C for 1 hour, followed by inactivation at 95°C for 5 minutes.

qPCR reaction setup

A qPCR master mix was prepared according to the manufacturer's guidelines for the target genes (spdC, *icaA*, *mecA*) and the reference gene (16S rRNA).

Thermocycler Conditions

Thermal cycling was conducted under the following optimized conditions: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles consisting of denaturation at 95°C for 20 seconds and annealing/extension at 60°C for 30 seconds. A final melt curve analysis was performed from 65°C to 95°C. These parameters were optimized using the Thermo Scientific™ Primer Annealing Temperature Calculator.

qPCR data analysis

Relative gene expression was calculated using the comparative CT (ΔΔCT) method (Livak & Schmittgen, 2001): ΔCT = CT (housekeeping gene) – CT (target gene)

Fold change = 2^(-ΔΔCT)

Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics software. Chi-square tests were applied at a significance level of $P < 0.01$, and one-way ANOVA followed by the Least Significant Difference (LSD) test was used at $P < 0.05$.

Results

Twelve *Staphylococcus aureus* strains were identified from a total of 30 stool samples collected from patients with diarrhea. The identification was based on cultural characteristics and biochemical tests, including Gram-positive staining, positive catalase and coagulase tests, negative oxidase test, and the appearance of yellow colonies on mannitol salt agar.

The antibiotic resistance profiles of the isolates were determined using the Vitek 2 system. The results demonstrated that the isolates exhibited varying degrees of resistance to multiple antibiotics, as detailed in Table 2.

Table 2 Antibiotics resistance of *S. aureus* strains.

Code of antibiotic	No. of isolates	No. of antibiotics
P & OX1	1 2 5 9 10	Resistant to (I) type of antibiotic
P, OX1 & CM	4 12	Resistant to (II) types of antibiotics
P, OX1, CM & TE	3 6 11	Resistant to (III) types of antibiotics
P, OX1, CM, TE & RA	7 8	Resistant to (IV) types of antibiotics

Expression analysis of spdC, mecA, and icaA genes

The expression patterns of the *spdC*, *mecA*, and *icaA* genes in Staphylococcus aureus strains with varying antibiotic resistance profiles were examined using RT-qPCR, as outlined in Table (2) and illustrated in Figure (1). The results revealed a striking upregulation of the *spdC* gene in strains categorized as type (IV) resistance—those resistant to four different antibiotic classes. Specifically, *spdC* expression reached 13.02 ± 0.89 in type IV strains, which was significantly higher

than in type I (1.66 ± 0.26), type II (1.90 ± 0.11), and type III (2.98 ± 0.36) strains (P < 0.001). Interestingly, while type IV strains stood out, the differences among types I, II, and III were not statistically significant (P > 0.05), suggesting that high *spdC* expression may be uniquely associated with broader antibiotic resistance.

Table 3 Gene expression of *spdC, mecA and icaA* in different antibiotic resistance types.

Groups	*spdC gene expression*	*mecA* gene expression	*icaA* gene expression
Type I	1.66 ± 0.26^{A}	1.74 ± 0.36^{A}	4.31 ± 0.72^{A}
Type II	1.90 ± 0.11^{A}	2.56 ± 0.46^{A}	14.20 ± 2.64^{B}
Type III	2.98 ± 0.36^{A}	2.20 ± 0.37^{A}	17.36 ± 4.81^{B}
Type IV	13.02 ± 0.89^{B}	5.03 ± 0.82^{A}	21.93 ± 4.49^{B}
p-value	0.001**	0.601	0.021**

Different letters indicate significant differences at $p < 0.05$.
SD: standard deviation; †: one way ANOVA; **: significant at P > 0.05

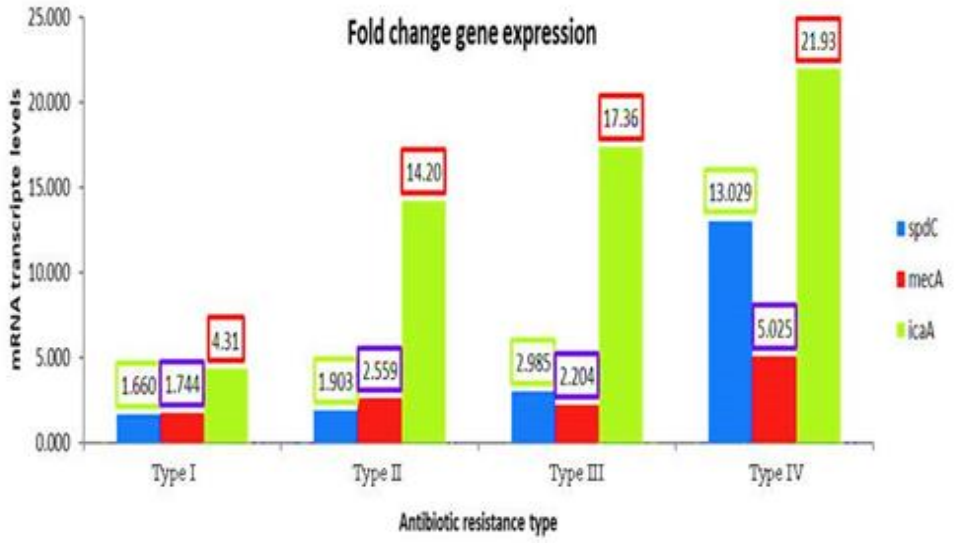

Fig 1. Fold change among different genes under investigation.

Similarly, expression of the *mecA* gene—a well-known marker of methicillin resistance—was elevated in type IV strains (5.03 ± 0.82), although this increase did not reach statistical significance when compared to type I (1.74 ± 0.36), type II (2.56 ± 0.46), or type III (2.20 ± 0.37) (P > 0.05). No significant differences in *mecA* expression were observed among the other resistance types either. This indicates that while *mecA* expression tends to rise with greater resistance, its variation across categories was less pronounced than that of *spdC*.

The real-time PCR amplification plots for these genes are presented in Figure 2 (A–D). The distinct color coding—yellow for *spdC*, green for *icaA*, blue for *mecA*, and red for 16S rRNA—helped visualize the amplification efficiency and gene activity levels across different isolates.

To ensure the accuracy and specificity of amplification, melting curve analysis was performed, as shown in Figure 3 (A–D). These curves display the change in fluorescence (RFU) as a function of temperature, helping identify specific DNA product formation without non-specific amplification. Figure 4

(A–D) further complements this by showing the melting peaks, where the derivative of fluorescence (–dF/dT) is plotted against temperature. Each gene retained its color code, allowing for easy comparison and confirmation of distinct amplification patterns.

Overall, the data point to a strong association between higher antibiotic resistance and increased expression of the *spdC* and *icaA* genes, particularly in multi-resistant *S. aureus* strains, while *mecA* expression showed a less consistent but still notable trend.

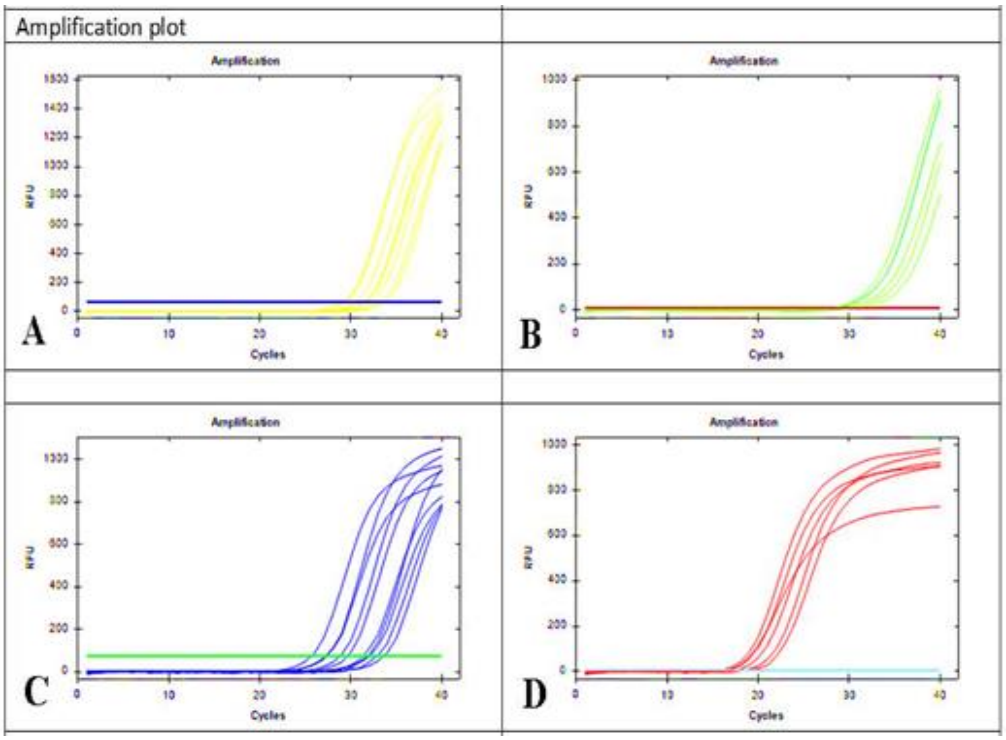

Fig. 2 (A, B, C, and D). Real-time PCR amplification plots for the *spdC, icaA, mecA*, and 16S rRNA genes in S. *aureus* isolates.

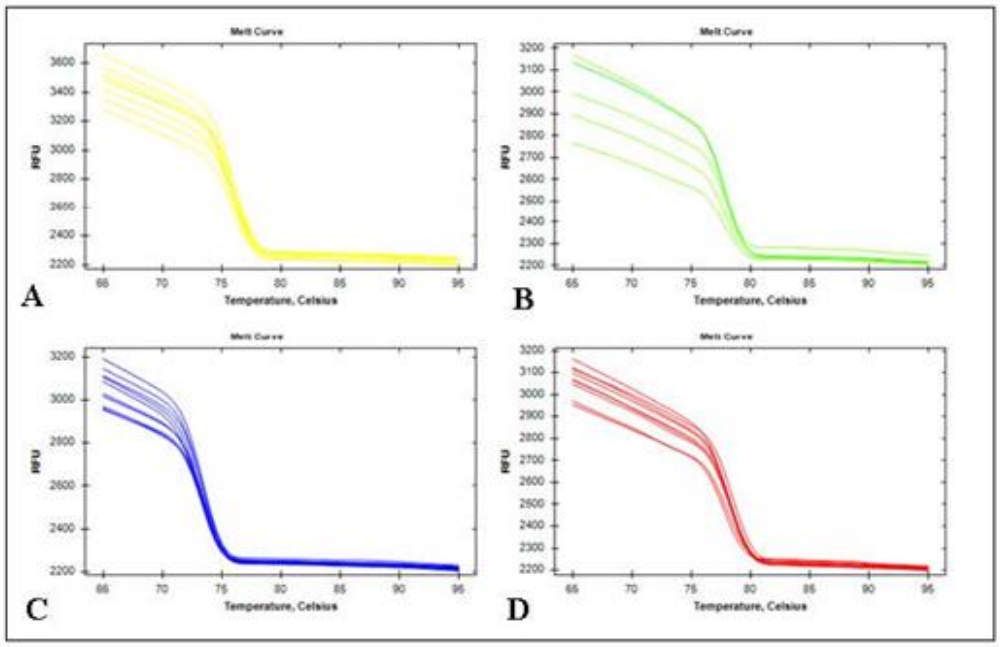

Fig. 3 (A, B, C, and D). RT-qPCR melt curve analysis of 16S rRNA, *spdC*, *icaA,* and *mecA* genes in *S. aureus* strains.

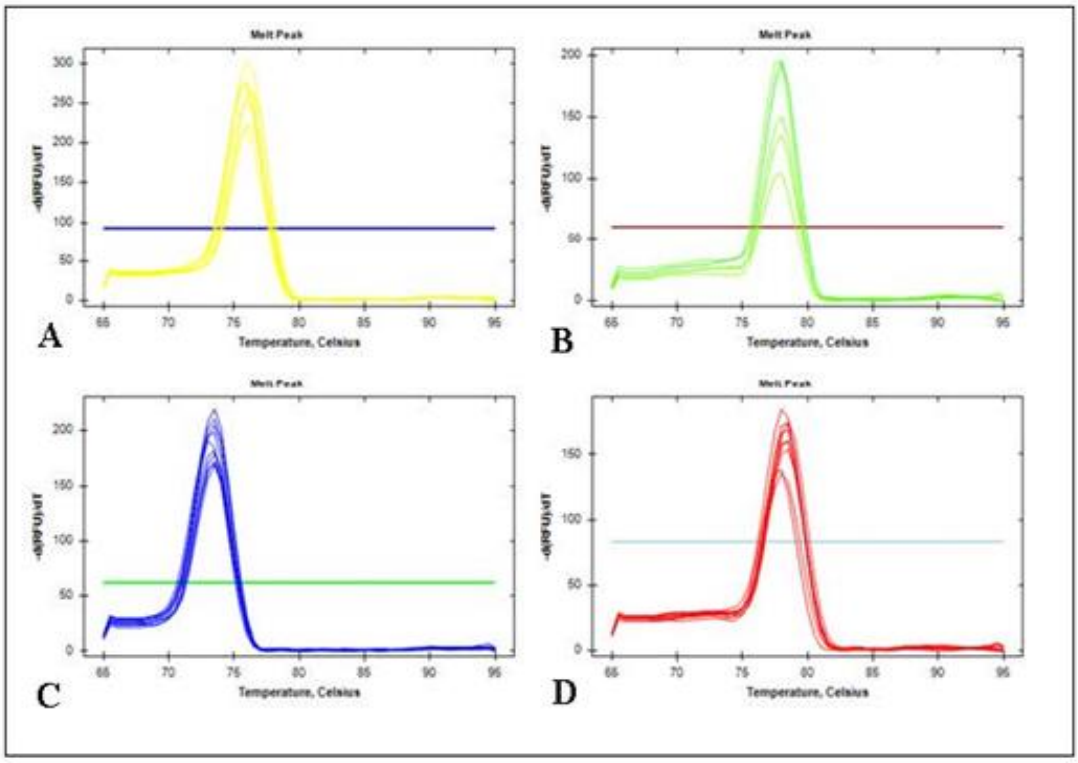

Fig. 4 (A, B, C, and D). Derivative melt peak curves from RT-qPCR showing the melting temperatures of amplified 16S rRNA, *spdC, icaA*, and *mecA* gene products in *S. aureus* isolates.

Discussion

S. aureus is a major human pathogen with a wide range of virulence factors that enable its survival and persistence in host environments (Liu, 2009). It has long been recognized as a leading cause of hospital-acquired infections (De Oliveira et al., 2020; Salem et al., 2024).

The current study identified *S. aureus* in 40% of samples from diarrhea patients, and all isolates displayed varying degrees of antibiotic resistance. Expression analysis revealed increased levels of the *spdC*, *mecA*, and *icaA* genes in isolates resistant to multiple antibiotic classes, particularly in those resistant to four different types (type IV).

The findings for *mecA* are consistent with those of Gharsallah et al. (2024), who reported the presence of *mecA* in 12 out of 30 clinical *S. aureus* isolates from various human samples in Tunisia. They also observed variation in gene expression levels between resistant and sensitive strains. Methicillin resistance rates vary by region, with high rates reported in countries such as the United States and China (Stefani et al., 2012).

However, the current study contrasts with Rezashateri et al. (2021), who found 100% of methicillin-resistant isolates from burn patients carried *mecA*, with high resistance rates to oxacillin and cefoxitin. Conversely, Juwita et al. (2022) found that among six resistant isolates, only one carried the *mecA* gene, suggesting possible alternative resistance mechanisms.

Regarding *icaA*, our results showed significantly increased expression in resistance types II, III, and IV, with no significant differences among them. This aligns with findings from Khalil & Al-Hayanni (2024), who reported 100% prevalence of *icaA* and *mecA* in isolates resistant to various antibiotics. Azmi et al. (2019) also reported 100% prevalence of *icaA* in MRSA strains.

Significantly elevated *spdC* expression in type IV resistance isolates supports the gene's role as a virulence regulator and potential contributor to antibiotic resistance, as previously suggested by Poupel et al. (2018). In contrast, Bakr et al. (2022) found inconsistent correlations between *spdC* expression and resistance, indicating that its role may vary depending on the context. Nevertheless, both studies indicate higher *spdC* expression in resistant strains compared to susceptible ones.

Some studies, such as Charfeddine et al. (Gharsallah et al., 2024), found no significant correlation between *mecA* and *spdC*, though they observed notable interactions between virulence genes and resistance markers. Additional research is needed to clarify the regulatory mechanisms linking *spdC*, *icaA*,

and *mecA* expression and to explore how these genes contribute to resistance and virulence in different infection contexts.

Due to the limited number of isolates obtained from diarrhea patients, further studies are necessary to confirm these findings and to investigate the broader role of *spdC* in antibiotic resistance and virulence regulation in *S. aureus* from diverse infection sources.

Conclusions

S. aureus strains isolated from diarrhea patients exhibited resistance to multiple antibiotic classes. This resistance was associated with increased expression of the *spdC* gene, a virulence factor and regulatory gene, alongside elevated expression of *icaA* and *mecA*. These findings underscore the growing public health threat posed by antibiotic-resistant *S. aureus* and highlight the need for strict antibiotic stewardship and strategic interventions to limit the spread of resistance.

Ethical Approval

This study was approved by the Research Ethics Committee of the College of Biotechnology, University of Al-Qadisiyah (Approval No. 1106, dated 20/5/2024), with verbal informed consent obtained from patients for sample collection under sterile conditions.

Conflict of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

We extend our sincere gratitude to the Faculty of Biotechnology, particularly the Molecular Biology Laboratory, for supporting this research. We also wish a swift recovery to all patients involved and express our appreciation to the reviewers for their valuable feedback.

References

Azmi K , Qrei W , Abdeen Z. (2019). Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of *Staphylococcus aureus* isolated from Palestinian patients. BMC genomics, 20, 1-12. https://doi.org/10.1186/s12864-019-5929-1

Bakr ME , Kashef MT, Hosny, AE.-DM, Ramadan MA. (2022). Effect of spd C gene expression on virulence and antibiotic resistance in clinical *Staphylococcus aureus* isolates. International Microbiology, 25(3), 649-659. https://doi.org/10.1007/s10123-022-00249-6

Cheung GY , Bae JS , Otto M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. Virulence, 12(1), 547-569. https://doi.org/10.1080/21505594.2021.1878688

Davis KA, Stewart JJ, Crouch HK, Florez CE, Hospenthal DR. (2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clinical Infectious Diseases, 39(6), 776-782. https://doi.org/10.1086/422997

De Oliveira DM , Forde BM, KiddTJ, Harris P N, Schembri MA , Beatson SA , Walker MJ. (2020). Antimicrobial resistance in ESKAPE pathogens. Clinical microbiology reviews, 33(3), 10.1128/cmr. 00181 - 00119. https://doi.org/10.1128/cmr.00181-19

Dubrac S , Bisicchia P , Devine KM , MsadekT. (2008). A matter of life and death: cell wall homeostasis and the WalKR (YycGF) essential signal transduction pathway. Molecular microbiology, 70(6), 1307-1322. https://doi.org/10.1111/j.13652958.2008.06483.x

Fetsch A, Johler S. (2018). *Staphylococcus aureus* as a foodborne pathogen. Current Clinical Microbiology Reports, 5, 88-96. https://doi.org/10.1007/s40588-018-0094-x

Frankel MB, Wojcik BM, DeDent AC, Missiakas DM, Schneewind O. (2010). ABI domain-containing proteins contribute to surface protein display and cell division in *Staphylococcus aureus*. Molecular microbiology, 78(1), 238-252.https://doi.org/10.1111/j.13652958.2010.07334.x

Gharsallah, C, Ferjani A, Ghariani M, FerjaniS , Kanzari L. Rehaiem A, Boutiba Ben Boubaker I. (2024). Impact of methicillin resistance on virulence factor expression in 1 *Staphylococcus aureus*: Insights from gene expression profiling. bioRxiv, 2024.2010. 2019.619075. https://doi.org/10.1101/2024.10.19.619075

Gründling A , Missiakas DM, Schneewind O. (2006). *Staphylococcus aureus* mutants with increased lysostaphin resistance. Journal of bacteriology, 188(17), 6286-6297. https://doi.org/10.1128/jb.00457-06

Haddad Kashani H, Schmelcher M, Sabzalipoor H, Seyed Hosseini E, Moniri R. (2018). Recombinant endolysins as potential therapeutics against antibiotic - resistant *Staphylococcus aureus*: current status of research and novel delivery strategies. Clinical microbiology reviews, 31(1), 10.1128/cmr. 00071 - 00017. https://doi.org/10.1128/cmr.00071-17

Hmed A, Salem M, Sofy A. (2024). Potential use of SAP-4 as a destructive and biofilm stripper against disobedient *Staphylococcus aureus* (SA) and methicillin-resistant S. aureus (MRSA) isolates. Microbial Biosystems, 9(2), 208-230. doi: 10.21608/mb.2024.307415.1136

Jabbar N, Abdul Wahid W. (2025). Effect of turmeric nanoparticles on gene expression of biofilm gene in Staphylococcus aureus. Microbial Biosystems, 10(1), 234-242. doi: 10.21608/mb.2025.297763.1108

Jin Z, Jiang Q , Fang B, Sun B. (2019). The ArlR-MgrA regulatory cascade regulates PIA-dependent and protein-mediated biofilm formation in Rbf-dependent and Rbf-independent pathways. International Journal of Medical Microbiology, 309(2), 85-96. https://doi.org/10.1016/j.ijmm.2018.12.006

Juwita S, Indrawati A, Damajanti R., Safika S, Mayasari NLPI. (2022). Multiple antibiotic resistance and virulence factors of *Staphylococcus aureus* strains isolated from dairy farms in South Sulawesi, Indonesia. Biodiversitas Journal of Biological Diversity, 23(2). 34-40. https://doi.org/10.13057/biodiv/d230244

Khalil WL, Al-Hayanni HS. (2024). Molecular Detection of the *mecA* and some Virulence Determinants in Methicillin-Resistant *Staphylococcus aureus*. Journal of the Faculty of Medicine Baghdad, 66(2), 247-253. https://doi.org/10.32007/jfacmedbagdad.6622282

Liana I, Astirin OP , Nugraheni ER. (2015). Antimicrobial activity of fraction from methanolic extract of senggani (Melastoma candidum) leaves towards *Staphylococcus aureus* and Sa. typhimurium and thin layer chromatography profile of the most active fraction. Asian Journal of Natural Product Biochemistry, 13(2), 66-77.

Liu GY. (2009). Molecular pathogenesis of *Staphylococcus aureus* infection. Pediatric research, 65(7), 71-77. https://doi.org/10.1203/PDR.0b013e31819dc44d

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Lynch JP, Zhanel GG. (2023). Escalation of antimicrobial resistance among MRSA part 1: focus on global spread. Expert Review of Anti-infective Therapy, 21(2), 99-113. https://doi.org/10.1080/14787210.2023.2154653

Millan B. (2016). Fecal Microbial Transplantation and its Expansion into the Treatment of Other Diseases. https://doi.org/10.7939/R3GQ6R939

Mohammadzadeh R, Mohammadi-Gollou A, Salehabadi Y. (2019). The Prevalence of CTX-M Beta-Lactamase Gene in Urinary Tract Infections in patients referring to Bonab city Hospitals. Iranian Journal of Medical Microbiology, 13(4), 302-309. http://dx.doi.org/10.30699/ijmm.13.4.302

Mokabel AM , Gebreel HM, Youssef HI, El-KattanN, Abdel-Wahhab MA. (2024). Prevalence of multidrug resistant bacteria in Egyptian hospitalized patients. Microbial Biosystems, 9(2), 121-132. https://doi.org/10.21608/mb.2024.312767.1147

O'Neill E, Pozzi C, HoustonP, Humphreys H, Robinson DA, LoughmanA, O'Gara JP. (2008). A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. Journal of bacteriology, 190(11), 3835-3850. https://doi.org/10.1128/jb.00167-08

Polívková M, Hubáček T, Staszek M , Švorčík V, Siegel J. (2017). Antimicrobial treatment of polymeric medical devices by silver nanomaterials and related technology. International journal of molecular sciences, 18(2), 419. https://doi.org/10.3390/ijms18020419

Poupel O, Proux C, Jagla B, Msadek T , Dubrac S. (2018). *SpdC*, a novel virulence factor, controls histidine kinase activity in *Staphylococcus aureus*. PLoS pathogens, 14(3), e1006917. https://doi.org/10.1371/journal.ppat.1006917

Procop G, Church D, Hall G, Janda W, Koneman E, Schreckenberger P, Woods G. (2017). The nonfermentative gram-negative bacilli. Koneman's Color Atalas and Textbook of Diagnostic Microbiology. Seventh edution, China, 316-431.

Rahman MT , Sobur MA, Islam MS, Ievy S, Hossain MJ, El Zowalaty ME, Ashour H M. (2020). Zoonotic diseases: etiology, impact, and control. Microorganisms, 8(9), 1405. https://doi.org/10.3390/microorganisms8091405

Rezashateri M, Ahrabi M, Salehi M. (2021). Molecular Analysis of the Presence of pvl, spa, and *mecA* Genes and Their Correlation with a Range of Antibiotics in *Staphylococcus aureus* Collected from Burn Patients. Iranian Journal of Medical Microbiology, 15(6), 625-637. https://doi.org/10.30699/ijmm.15.6.625

Salem MS, HmedA A , Sofy AR. (2024). Potential use of SAP-4 as a destructive and biofilm stripper against disobedient *Staphylococcus aureus* (SA) and methicillin-resistant *S. aureus* (MRSA)

isolates. Microbial Biosystems, 9 (2) . https://doi.org/10.21608/mb.2024.307415.1136

Sedarat Z,Taylor-Robinson AW. (2019). A Consideration of antibacterial agent efficacies in the treatment and prevention of formation of *Staphylococcus aureus* biofilm. Journal of Microbiology and Infectious Diseases, 9(04), 167-172. https://doi.org/10.5799/jmid.657903

Sila J, SauerP, Kolar M. (2009). Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, panton-valentine leukocidin and tsst-1 between methicillin-resistant and methicillin-susceptible isolates of *Staphylococcus aureus* at the university hospital in olomouc. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 153(3), 215-218.

Stefani S, Chung DR, Lindsay JA, Friedrich A W, Kearns AM, Westh H, MacKenzie FM. (2012). Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. International journal of antimicrobial agents, 39(4), 273-282. https://doi.org/10.1016/j.ijantimicag.2011.09.030.