Role of *murA* and *fosB* genes in Fosfomycin Resistance in *Staphylococcus aureus* isolated from Urinary tract infection patients

Shehab Khalid Shehab* and Rana Kadhim Mohammed
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq
*Corresponding author E-mail: shehab.khaled2302@sc.uobaghdad.edu.iq

Abstract
Bacterial infection is difficult to treat due to the presence of resistant pathogenic. This study aimed to investigate the phenotypic and molecular aspects of fosfomycin resistance as indicated by the modifying enzymes FosB and MurA in *Staphylococcus aureus* isolates from patients with Urinary tract infections (UTIs). This study isolated and identified *S. aureus* isolates from urine specimens using morphological characteristics of the biochemical tests, culture media, and genotypic identification using the *16S rRNA* gene. Investigation of antimicrobial susceptibility for eleven different antimicrobials. The study examined the antibacterial properties of Fosfomycin using minimum inhibitory concentration (MIC). Also, used molecular assays to identify, sequence, and expression levels of Fosfomycin resistance genes (*murA*, and *fosB*) under different treatments. The identification methods of *S. aureus* showed 50 isolates. Antimicrobial susceptibility testing revealed a significant prevalence of resistance to various antimicrobials. In comparison to the breaking point, the number of *S. aureus* isolates showed MIC concentration of more than 128 μg/ml was 24% (12 isolates) and appeared resistant to Fosfomycin. The molecular assays reveal the genetic aspects of resistance and its modification, with the presence of Fosfomycin resistance *murA*, and *fosB* genes and an increase in gene folding expression under different treatments.

Keywords: Fosfomycin; *S. aureus*; MurA; FosB; Urinary tract infection.

Introduction

The urinary system is one of the most vital systems in the animals and the human body (Drolet, 2019). When one or more urinary tract organs are invaded and infected, it's known as a Urinary tract infection (UTI). UTIs are caused by bacteria that originate from the gastrointestinal tract, colonize the external genitalia, invade the bladder and urethra, and obstruct the flow of urine (Hydronephrosis), these bacteria can also be originated from external sources, such as unhygienic practices, the normal flora of the genital areas, skin, and anus (Mohammed *et al*., 2020).

Staphylococcus aureus is the major cause of multiple infections ranging from superficial skin infections to deeper infections of hair follicles and deep tissue infections, systemic infections including lung, blood, and bones (Ondusko and Nolt., 2018). One of these agents that can cause an infection and infiltrate the urinary tract is *S. aureus*. However, an infection can become seriously life-threatening if left untreated (Chalise, 2021). In general, Gram-positive bacteria especially *S. aureus* have become important sources of hospital- and community-acquired infections because they are nearly immune to medications and spread readily (Lunacek et al., 2014).

Fosfomycin, with a unique mode of action, a derivative of phosphonic acid, prevents the creation of UDP-N-acetyl glucose amine enolpyruvyl transferase (MurA), an enzyme crucial to the biosynthesis of peptidoglycans, thereby irreversibly inhibiting the synthesis of bacterial cell walls

(Shan et al., 2020). Fosfomycin resistance can arise from several causes, such as enzymatic inactivation, target enzyme impairment, and decreased antibiotic permeability (Zheng et al., 2022).
The antimicrobial fosfomycin targets the important enzyme MurA, attaching to the protein irreversibly to render it inactive. The antimicrobial fosfomycin-binding site mutation in MurA causes resistance to it (El-Khoury et al., 2022).
The most important mechanism in Fosfomycin resistance development is the enzymatic inactivation associated with *fos* genes. One of the *fos* genes types is *fosB* (FosB is a thiol-S-transferase) which confers resistance to fosfomycin in wide chromosomes and plasmids of many Gram-positive bacteria, including *E. faecium, S. epidermidis, S. aureus, Bacillus cereus, B. anthracis, B. subtilis* (Mosime, 2021).
The aim of this study is the molecular study of the essential enzyme in peptidoglycan biosynthesis *murA* and Modifying-enzyme *fosA* genes as Fosfomycin resistance in *S. aureus*.

Materials and methods

Samples Collection and Diagnosis

Urine specimens were collected from patients with underdiagnosed UTIs and subsequently transported in a sterile container to the laboratory. Specimens of urine were cultured aseptically on blood agar and BHI agar plates and then incubated at 37 °C overnight. Gram stain, biochemical assays (catalase, oxidase, and coagulase), and genotypic identification utilising the *16S rRNA* gene were among the common laboratory procedures used to identify *S. aureus*.

Susceptibility test

The Kirby-Bauer disc diffusion method was used to test *S. aureus* antibiotic susceptibility by the guidelines set out by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2023) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2022). The Ampicillin (AMP10), Ceftaroline (CPT30), Oxacillin (OX30), Gentamicin (GEN10), Trimethoprim (TR5), Trimethoprim-sulfamethoxazole (SXT1.25/23.75), Vancomycin (VA30), Nitrofurantoin (NIT300), Ciprofloxacin (CIP5), Norfloxacin (NX10), and Tetracycline (TE30) were used. By measuring and interpreting the zone of inhibition surrounding each disc as sensitive, moderate, or resistant based on CLSI guidelines,

Minimum Inhibitory Concentration (MIC)

The MIC for Fosfomycin was determined for all the *S. aureus* isolates by the CLSI (2023) criteria by a standard two-fold serial dilutions method, with serial dilution of Fosfomycin concentrations values ranging from 16 to 1024 µg/ml. Bacterial suspension was prepared were adjusted to McFarland turbidity (0.5) equal to 10^8 Cells/ml. The MIC outcome was read overnight according to the lowest concentration of antibiotics in which no visible growth of the microorganism occurs is considered the MIC of the given isolates (Majeed and Al-Aubydi, 2019).

DNA Extraction

From a 1ml culture, chromosomal DNA was extracted using a Presto ™ Mini gDNA Bacteria Kit (Geneaid, Thailand). Chromosomal DNA, after purification, was kept at -20°C. A nanodrop device was used to measure DNA concentration and purity. One µl of each DNA sample was used to measure optical density (O.D) at 260 nm and 280 nm wavelengths.

Genotyping detection and diagnosis

Conventional polymerase chain reaction (PCR) was performed to amplify different fragments of the genes under study and identify *S. aureus* and resistance-associated genes. Conventional PCR was

used to amplify the following: *16S rRNA* gene to Identify the gene of *S. aureus*. The *murA* gene, and *fosB* gene*:* resistances genes of *S. aureus.*

Thermal Cycler; PCR Technique

Primers were used in the PCR to amplify particular DNA fragments (Table 1). Three microliters of template DNA, ten microliters of GoTaq®Green Master Mix, and one microliter of each of the forward and reverse primers (10poml)) were present in the reaction mixture. The addition of deionized nuclease-free water brought a PCR mixture up to 20 µl. For the uniplex PCR of every gene, the parameters specified in Table 2 were followed. Gradient PCR was used to optimize the PCR program's temperature and duration. Using a gel image analysis apparatus and a UV light source, the PCR products were found after the samples were electrophoresed on 1.5% (w/v) agarose (Promega, USA) in 1X TBE buffer stained with a safe stain.

All tested (*16S rRNA*, *murA*, and *fosB*) genes had uniplex PCR products that were kept at -20 ºC. After that, nucleotide sequencing was done by sending 25µl of PCR product sample and primer (Forward) to NICEM Company in the USA. The National Centre for Biotechnology Information (NCBI) received the data from gene sequencing and processed it using geneious software (version 10. prime), comparing the results with the NCBI control strain.

Table 1. Primer sequences used in this study.

Genes	Primers	Nucleotide sequences (5´ to 3´)	PCR product (bp)	Reference
16S rRNA	Forward	ACGGTCTTGCTGTCACTTATA	257	Johnson *et al*., 2016
	Reverse	TACACATATGTTCTTCCCTAATAA		
murA	Forward	GCCCTTGAAAGAATGGTTCGT	1600	Fu et al., 2016
	Reverse	GTTACAATACTCGACGCAGGT		
fosB	Forward	TTGCAGGCCTATGGATTGCT	247	Designed in this study
	Reverse	CTGTTCTCAAGTGTGCCAGT		

Table 2. Program condition for uniplex PCR amplification of each gene in this study.

Gene	Initial denaturation (ºC/min)	No. of cycle	Denaturation (ºC/min)	Annealing (ºC/min)	Extension (ºC/min)	Final extension (ºC/min)
16S rRNA	95/5	35	94/1	52/1	72/1	72/10
murA	95/5	35	94/1	57/1	72/1	72/10
fosB	95/5	35	94/1	55/1	72/1	72/10

Gene Expression

Gene expression levels were measured using Real Time-PCR in order to detect the behaviours of specific components related to the resistance of *S. aureus* isolates following stress with doses of fosfomycin at 512µg/ml and 1024µg/ml.

The current investigation examined the gene expression of ten isolates of pathogenic *S. aureus* that were resistant to fosfomycin. The target genes (*murA*, and *fosB*), which are thought to be essential chromosomal resistance factors for fosfomycin in *S. aureus*, were expressed in each isolate using the purified RNA that was extracted from it. Real-time PCR was used to calibrate and normalize each gene's expression level to that of the housekeeping gene *S. aureus 16S rRNA* (Al-Hayali et al., 2023). We used a commercial RNA extraction kit to extract RNA from bacterial cultures.

To achieve the final volume of 20µL, the one-step RT-PCR reaction mixture components were added to the template (3µl), primers (Table 3) [1µl from each of the forward primer and reverse

primer (10poml)], q PCR Master Mix (10µl), and deionized nuclease-free water. There were two steps involved in setting the reaction conditions (Table 4). All templates were run in triplicates.

Table 3. Primer sequence used in this study.

Gene	Primer	Nucleotide sequence (5´ to 3´)	PCR product bp	Reference
16S rRNA	Forward	CGGAAGATTCCCTACTGCTG	111	Designed in this study
	Reverse	TAACGGCTTACCAAGGCAAC		
murA	Forward	TGATTGCATATCAGTCGGGA	122	Designed in this study
	Reverse	AAGAAATGGGCGTTGAATTG		
fosB	Forward	TGCAGGCCTATGGATTGCTT	114	Designed in this study
	Reverse	TGCCAATATTTAAATTCGCTGTCA		

Table 4. Program conditions of qRT-PCR.

Gene	RT. Enzyme Activation (ºC/min)	Initial denaturation (ºC/min)	No. of cycle	Denaturation (ºC/min)	Annealing (ºC/min)	Extension (ºC/min)
16S rRNA	37/ 15	95/ 5	40	95/ 0.5	56/0.5	72/0.5
murA	37/ 15	95/ 5	40	95/ 0.5	52/0.5	72/0.5
fosB	37/ 15	95/ 5	40	95/ 0.5	57/0.5	72/0.5

Statistical Analysis

One-way ANOVA tests were used for statistical analysis of the mean±standard deviation, and GraphPad Prism 8 and SPSS 25 were used for statistical analysis of the Chi-square ($\chi 2$) test on qualitative data. A $P \leq 0.05$ p-value was considered statistically significant (Bland, 2015).

Results and Discussion

Identification of *S. aureus*

The *S. aureus* was identified based on morphological characteristics of culture media, biochemical tests, and genotypic identification to detect the *16SrRNA* gene in all isolates, which allowed for the genotypic identification of *S. aureus*. Based on the amplified size of 257 bp and *16S rRNA*, the results demonstrated that all isolates were *S. aureus* in 100% of the cases. As compared to the DNA ladder (100pb), Figure 1 shows the bands of the positive results.

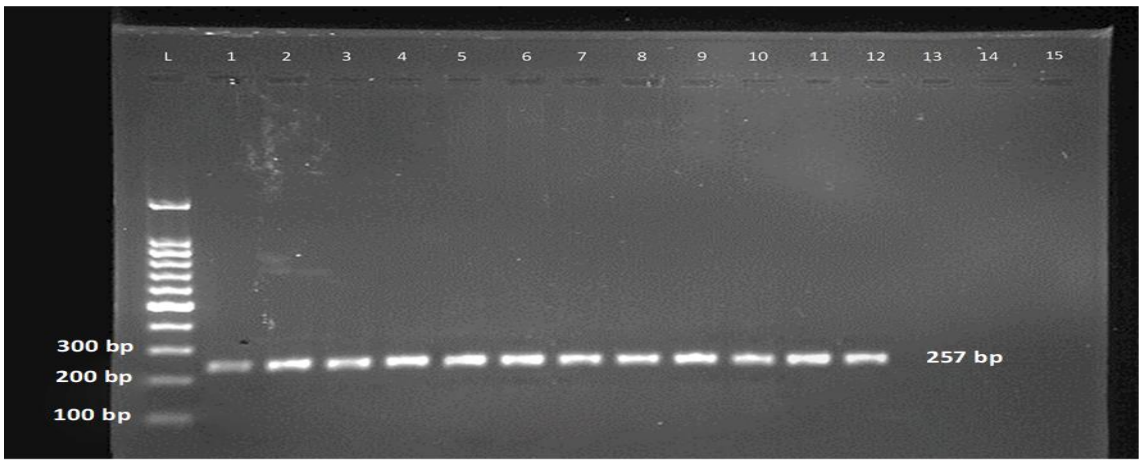

Figure 1. Agarose gel electrophoresis of amplified *16S rRNA* (257 bp) gene for *S. aureus* using conventional PCR. The 1.5% agarose, 7 V/cm^2 for 80 min, was stained with safe stain dye and visualized by a UV transilluminator. Lane L: 100 bp DNA ladder. Lanes 1-12: Amplicons *16S rRNA* gene for *S. aureus*. Lanes 13-14: Control: DNA extracted from different bacterial isolates (*S. epidermidis* and *S. pneumoniae*). Lane 15: Negative control (containing all PCR mixtures with water as a template).

The stability of the *16S rRNA* gene over time and its conservation make it one of the most important factors in this categorization (Matsuo et al., 2021). This gene might be used in diagnosis and typing after routine diagnostic approaches are exhausted because it contains highly conserved regions among all bacterial species, as well as variable regions within different bacterial genera, providing a unique sequence for each species (Hutchison et al., 2016). Also, molecular diagnosis applies the *16S rRNA* gene as the final diagnosis for their bacteria, which was confirmed by Omar and Mohammed, 2021; Ibrahim and Mohammed, 2023), as the final diagnosis for their bacteria.

Susceptibility testing

In this study, *S. aureus* isolates showed diverse and varied susceptibility patterns to antimicrobials of different antimicrobial classes. Table 5 shows the susceptibility test results for twelve different antimicrobials.

Table 5. Percentage results of antimicrobial susceptibility tests against *S. aureus* isolates.

Antimicrobial class	Antimicrobial	Resistance		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
β-Lactams	Ampicillin	50	100	0	0	0	0
	Ceftaroline	40	80	0	0	10	20
	Oxacillin	33	66	0	0	17	34
Aminoglycosides	Gentamicin	25	50	4	8	21	42
Folate pathway antagonists	Trimethoprim	48	96	0	0	2	4
	Trimethoprim-sulfamethoxazole	42	84	4	8	4	8
Glycopeptides	Vancomycin	10	20	0	0	40	80
Nitroheterocyclics	Nitrofurantoin	13	26	6	12	31	62
Quinolones	Ciprofloxacin	37	74	6	12	7	14
	Norfloxacin	41	82	6	12	3	6
Tetracyclines	Tetracycline	29	58	9	18	12	24

In this research, we investigated how quickly *S. aureus* isolates from patients with UTIs developed drug resistance. Resistance to β-lactams: In *S. aureus*, the two-way functional transglycosylase-transpeptidase PBP2 is the most often occurring inhibitory target site for β-lactam antimicrobials. The transglycosylase-containing region of the enzyme is responsible for coordinating the transfer of peptidoglycan's disaccharide pentapeptide raw material from membrane-bound lipid II to budding polysaccharide chains. The transpeptidase-containing domain helps join the glycine cross-bridge of the chain's fourth d-alanine next to it (Walsh and Wencewicz, 2016). This class of antibiotics comprises cephalosporin, methicillin, oxacillin, and penicillin. By attaching to and deactivating the penicillin-binding proteins in the bacterial cell wall, they prevent the transpeptidation stage of peptidoglycan production (Page, 2012).

The mechanism of resistance common to all aminoglycoside medications involves the synthesis of an enzyme known as aminoglycoside-modifying enzyme (AME), which chemically alters the drug (usually by transferring an acetyl group), hence reducing its binding affinity for the 30S ribosomal subunit. The *aac*, *ant*, and *aph* genes are potential candidates. It is believed that plasmids are the source of these genes (Dworzack, 2019).

The *tetK* gene, which causes active drug efflux, and the *tetM* gene-encoded ribosomal protection protein (RPP), which competitively binds to the 30S subunit, are the two processes by which tetracycline resistance arises. The medication cannot attach to the RPP while it is bound. It is believed that plasmids are the source of both of these systems (Ogawara, 2019).

Resistance to *S. aureus* has grown owing to the widespread use of quinolones and fluoroquinolones for treating UTIs worldwide. The mechanism of action of fluoroquinolone medications is the inhibition of gyrase or topoisomerase IV, which stops transcription and DNA replication. Mutations in the GrlA subunit of topoisomerase IV (encoded by the *grlA* gene) or the GyrA subunit of gyrase (encoded by the *gyrA* gene) cause resistance to these medications by reducing their capacity to bind to their targets. In addition, the stimulation of the NorA efflux pump (*norA* gene) results in some mild resistance to norfloxacin (and possibly ciprofloxacin). (Hooper and Jacoby, 2016).

In the *dhfr* (dihydrofolate reductase enzyme) gene with reduced binding of trimethoprim resistance mutates (Wróbel et al., 2020). The resistance of *S. aureus* to trimethoprim-sulfamethoxazole due to chromosomal mutations (often single point mutations) in the *dhps* (dihydropteroate synthase enzyme) gene that allows pABA binding but greatly reduces binding of sulfamethoxazole. Is commonly the cause of resistance to these antimicrobials (Sánchez-Osuna et al., 2020).

Minimum Inhibitory Concentration (MIC)

The MIC was identified as the lowest concentration of antimicrobial agent which inhibited visible growth. The isolate will be considered resistant if it stands greater than the breaking point already fixed by CLSI (2023) breakpoint for fosfomycin equal to or less than 64µg /ml is considered sensitive, 128 µg /ml is considered intermediate, while equal or more than 256 µg /ml considered resistant. Table 6 represents the MIC values registered in the current study and some of the *S. aureus* isolates recorded MIC concentrations of more than 128 *µ*g/ml (12 isolates).

Table 6. The MIC of fosfomycin values as recorded against *S. aureus*.

No.	Fosfomycin concentration (*µ*g/ml)	No. of resistance *S. aureus* isolates (n =50)	N (%)	Result
1	16	50	100	Sensitive
2	32	40	80	Sensitive
3	64	25	50	Sensitive
4	128	16	32	Intermediate

5	256	12	24	Resistant
6	512	8	16	Resistant
7	1024	5	10	Resistant

The MIC values for fosfomycin registered in our study, the number of *S. aureus* isolates recorded MIC concentration of more than 128 μg/ml was 24% (12 isolates). The MIC of Fosfomycin for *S. aureus* in some studies, the study in China, out of 67 *S. aureus* isolates, 8 (11.9%) were fosfomycin resistant (MIC $\geq$ 256 µg/mL) (Fu et al., 2016). In a study evaluating *S. aureus* clinical isolates, identified Fosfomycin MICs found that 30/70 (42.8%) of isolates were resistant (Campanile et al., 2020). Chen *et al*. (2022) illustrated that some *S. aureus* (19.4%) isolates were resistant to fosfomycin and high MIC was observed against these isolates. In contrast, research done by Truong-Bolduc *et al*., (2018), and Saravolatz and Pawlak (2023) were reported 100% of isolates were sensitive to fosfomycin, this studies in turn not compatible with this study.

Detection of Fosfomycin resistance-related genes

Fifty *S. aureus* isolates were tested for Fosfomycin resistance (*murA* and *fosB*) genes using a PCR method.

- The *murA* gene

The amplified *murA* gene (1600 bp) for all DNA samples from 50 *S. aureus* isolates. This detection yielded a positive result for all 50/50 (100%) isolates for the *murA* gene, as shown in Figure 2.

The results of *murA* gene sequences of 5 *S. aureus* isolates resistance to fosfomycin. Table 7 shows seven distinct mutations detected in the *murA* gene throughout compared with the reference NCBI database (NC_002745). Were observed with 4 missense mutations (Type A to Type D) which resulted in indistinct amino acid substitutions within the MurA protein (Leu 58 Ser, Thr 110 Ile, Pro 272 Thr, and Gly 285 Cys), while each of the remaining mutations non-change in amino acid (silent mutation).

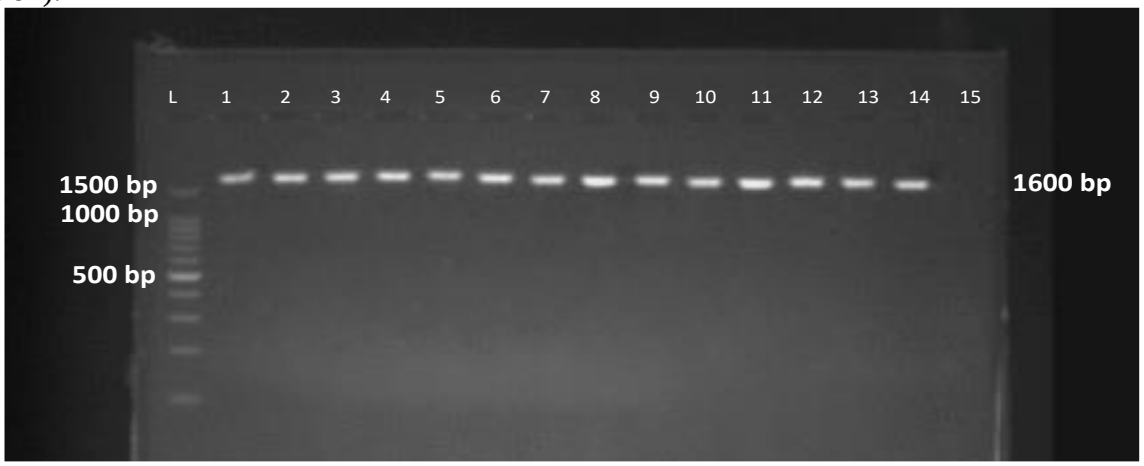

Figure 2. Agarose gel electrophoresis of amplified *murA* (1600 bp) gene from *S. aureus* using conventional PCR. The 1.5% agarose, 7 V/cm^2 for 80 min, was stained with safe stain dye and visualized by a UV transilluminator. Lane L: 100 bp DNA ladder. Lanes 1-14: Amplicons *murA* gene for *S. aureus*; all lanes represent positive results. Lane 15: Negative control (replacement of the DNA template with water in the PCR mixture).

Table 7. Characteristics of *S. aureus* isolates contained *murA* gene mutations.

No.	Mutation No.	Isolates No.	Nucleic acid Substitution	Amino acid Substitution	Type of Mutations
1	Type I	S12, S23, S24, S26, and S31	T105A	Ile 35 Ile	Silent
2	Type A	S12, and S31	T173C	Leu 58 Ser	Missense

3	Type B	S12, S23, and S26	C329T	Thr 110 Ile	Missense
4	Type II	S12, S23, S24, S26, and S31	T351G	Leu 117 Leu	Silent
5	Type C	S12, S23, S26, and S31	C814A	Pro 272 Thr	Missense
6	Type D	S12, S23, and S26	G853T	Gly 285 Cys	Missense
7	Type III	S12, S23, and S24	T1065G	Leu 355 Leu	Silent

- The *fosB* gene

The amplified of *fosB* gene (247 bp) for all DNA samples from 50 *S. aureus* isolates. This detection yielded a positive result for 5/50 (10%) isolates that had bacillithiol-S transferases *fosB* resistance gene with the molecular size of amplified products, as shown in Figure 3. According to the results of sequencing and data analyzing by geneious software the identity reached 100% for *fosB*.

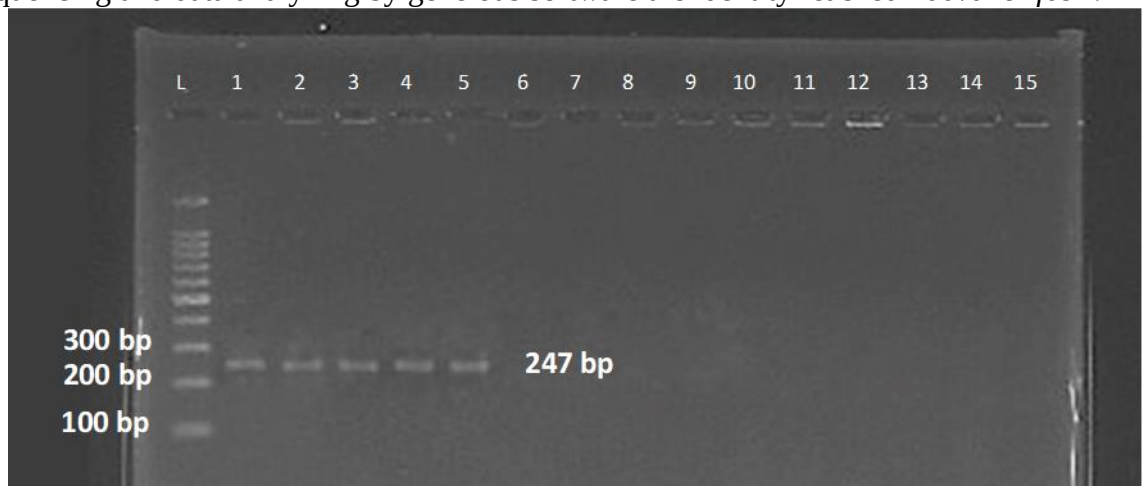

Figure 3. Agarose gel electrophoresis of amplified *fosB* (247 bp) gene from *S. aureus* using conventional PCR. The 1.5% agarose, 7 V/cm^2 for 80 min, was stained with safe stain dye and visualized by a UV transilluminator. Lane L: 100 bp DNA ladder. Lanes 1-14: Amplicons *fosB* gene for *S. aureus*, (A) lanes 1 to 5 represent positive results. Lane 15: Negative control (replacement of the DNA template with water in the PCR mixture).

Determination of Gene Expression Level by RT-PCR

In the present study, the expression of the targeted Fosfomycin resistance included transporter (*murA* and *fosB*) genes was done for five isolates under two treatments of fosfomycin MIC concentration (T1: 512 and T2: 1024 µg/ml) and compared with the control untreated (without antibiotic).

The *16SrRNA* gene was referred to as the housekeeping gene for all other genes. Such practice has been discouraged since it is evident that some housekeeping genes could vary to a larger extent in specific biological samples The result of *16SrRNA* for this study showed Ct values ranging from 19 to 20.01.

The effect of Fosfomycin on the expression of *murA* and *fosB* in *S. aureus* isolates is shown in Figure 4 and 5, respectively. The analysis of *murA* gene expression in comparison with the control group revealed a notable significant increase in gene folding expression across all isolates, for all treatment statuses. The Ct values corresponding to the *murA* gene under all treatment conditions provided insights into the initial mRNA content of the specimens. The data indicated that the concentrations of fosfomycin were influencing and correlated with the excellent value of Ct in all treatment conditions, which suggests an increase in gene folding expression.

The analysis of *fosB* gene expression in comparison with the control group revealed a notable significant increase in gene folding expression across all isolates for all treatments. The Ct values corresponding to the *fosB* gene under all treatment conditions provided insights into the initial mRNA content of the specimens. The data indicated that the concentrations of fosfomycin were

influencing and correlated with the excellent value of Ct in all treatment conditions, which suggests an increase in gene folding expression.

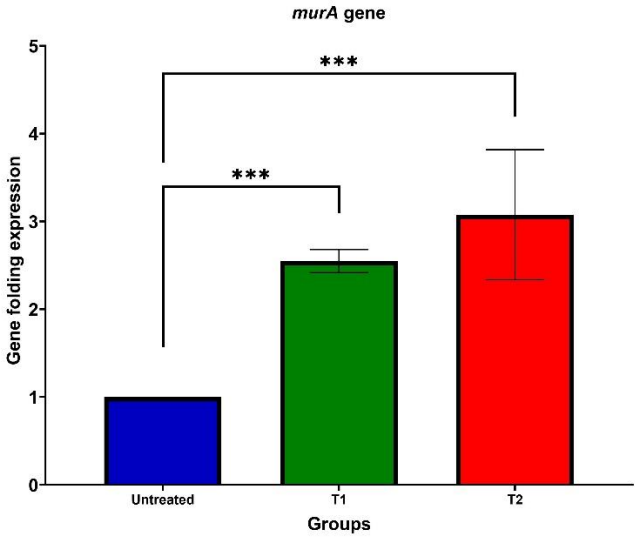

Figure 4. The Mean gene folding expression for *murA* in different statuses. T1: fosfomycin at 512 µg/ml, T2: fosfomycin at 1024 µg/ml.

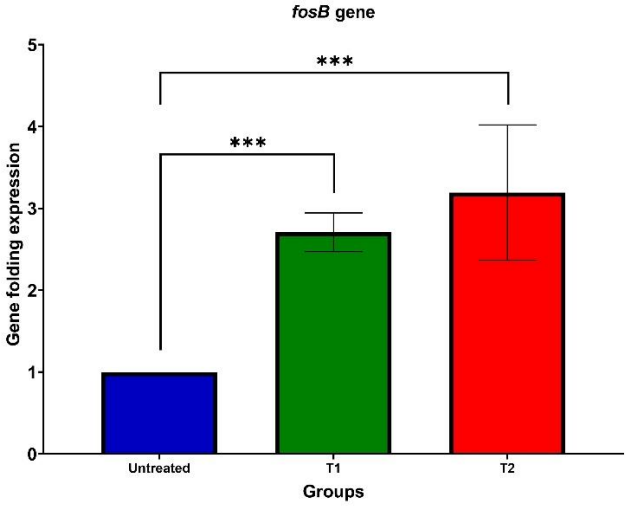

Figure 5. The Mean gene folding expression for *fosB* in different statuses. T1: fosfomycin at 512 µg/ml, T2: fosfomycin at 1024 µg/ml.

Altering the antimicrobial MurA target is a typical mechanism linked to fosfomycin resistance. Using this technique, the antibiotic binds to the protein irreversibly, making the enzyme inactive (Dos Santos et al., 2021). The enzymatic activity of MurA is vulnerable to inhibition by fosfomycin in a dose-dependent manner. The presence of an aspartic acid (Asp) residue in the catalytic region of MurA proteins has been observed in specific pathogenic bacteria, including *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Vibrio fischeri*, and *Borrelia burgdorferi*. Mutations in the Asp, Cys115, and Glu residues enable MurA to exhibit inherent resistance to fosfomycin (Xin et al., 2022). Accordingly, in microbiological and molecular investigations, the *murA* gene is a useful target for determining fosfomycin resistance and possible inhibitors (Raina et al., 2021). It is exceedingly unlikely that *murA* will stop functioning because it is necessary for the creation of cell walls. According to earlier research, the majority of *murA* mutant isolates also had comparatively low MICs and transporter gene alterations (Liu et al., 2020). Nevertheless, mutagenesis has not yet

been conducted, and the underlying mechanism remains unexplored. Hence, it is plausible that the *murA* mutation might manifest as a polymorphism, and alternative pathways may also contribute to the development of resistance (Wang et al., 2022).

The *murA* expression levels among fosfomycin-sensitive and fosfomycin-resistant *S. aureus* are different. This is contrary to many other previous findings that reported the overexpression of the *murA* gene to significantly raise MIC for fosfomycin (Olesen et al., 2014). Besides, the overexpressing of target enzymes, MurA, also led to resistance by fosfomycin in *S. aureus* (Truong-Bolduc *et al*., 2018). The other study by Xu *et al*. (2020) found that when comparing the expression of *murA* between *S. aureus* ATCC 29213 and fosfomycin-resistant and fosfomycin-sensitive strains, there was a significant difference.

The alteration of *murA* has been shown to confer resistance to fosfomycin. The affinity for fosfomycin binding and its activity are reduced by amino acid alterations in the active region of MurA. Fosfomycin inhibits peptidoglycan production through covalent interaction with the cysteine-115 residue of MurA. Resistance to Fosfomycin occurs when the mutation occurs in an active site of MurA, specifically, a substitution of cysteine with aspartate (Zheng et al., 2022). Another study showed that *murA* overexpression resulted in the acquisition of resistance to fosfomycin (Turner et al., 2020).

One of the three resistance pathways is linked to the inactivation of fosfomycin through either phosphorylation of the phosphonate group or enzymatic disruption of the oxirane ring found in the antibiotic. Numerous enzymes, including fosfomycin-specific epoxide hydrolase (FosX), L-cysteine thiol transferase (FosB), and glutathione transferase (FosA), aid in the opening of the oxirane ring (El-Khoury et al., 2022). The cofactor for these bacillithiol-Stransferases is Mg2+. The chromosomally placed FosB of *S. aureus* is in charge of the innate level of fosfomycin activity; deletion of the bacillithiol synthesis machinery or the *fosB* gene significantly increases the sensitivity to fosfomycin (Silver, 2017).

In the study by Aiezza et al. (2023), some *S. aureus* clonal lineages have a resident chromosomal *fosB* gene, which they can overexpress to develop fosfomycin resistance. According to previous studies, the acquisition of FOM-modifying enzymes, which are encoded by *fos* genes, has been found to lead to resistance, specifically exceeding the established breakpoints, against fosfomycin (Falagas et al., 2019). The extra cytoplasmic sigma factor SigW, a regulator that plays a significant role in producing inducible resistance to antimicrobial drugs, is necessary for the expression of FosB. According to the information that is currently available, the *fosB* gene is either plasmid- or chromosomally encoded and is primarily expressed in Gram-positive bacteria (Falagas et al., 2019).

Conclusions

This study investigated the prevalence of fosfomycin resistance in *S. aureus* for the first time in Iraq. The highest incidence resistance to several antimicrobials in *S. aureus* clinical isolates. Furthermore, the molecular assays examining genomic Fosfomycin resistance included *murA* and *fosB* genes. In our study, *S. aureus* isolates found the mutation in the *murA* gene in isolates resistant to fosfomycin, while non-found mutation with *fosB* gene. Their expression levels under different treatments shed light on the genetic aspects of resistance and its modulation. The presence of these resistance genes in all isolates, combined with increased gene expression changes in response to treatments, adds depth to our understanding of resistance mechanisms.

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