

Gene Expression of Carbapenemase-Encoding Genes in Multi-Drug Resistant Staphylococcus aureus from Clinical Sources

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Received: March 22, 2024; Accepted: Apr 29, 2024; Published: May 10, 2024;

Abstract: The emergence of multi-drug resistant (MDR) Staphylococcus aureus strains, particularly those expressing carbapenemase genes, poses a significant challenge in clinical settings. This study investigates the prevalence and expression profiles of carbapenemase genes (blaVIM, blaOXA-143, blaGES, blaOXA-23, and blaOXA-24) in S. aureus isolates from burn and wound patients in Tehran hospitals. A cross-sectional study was conducted using 100 S. aureus samples, which underwent biochemical testing for identification and antibiotic susceptibility testing. Gene expression analysis was performed using real-time PCR, and the results were analyzed with GRAPH PAD PRISM software. The study highlights the importance of understanding the molecular mechanisms of antibiotic resistance in S. aureus and contributes to the development of effective treatment strategies against MDR infections.

Keywords: S. aureus, antibiotic resistance genes, gene expression, MDR



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Introduction

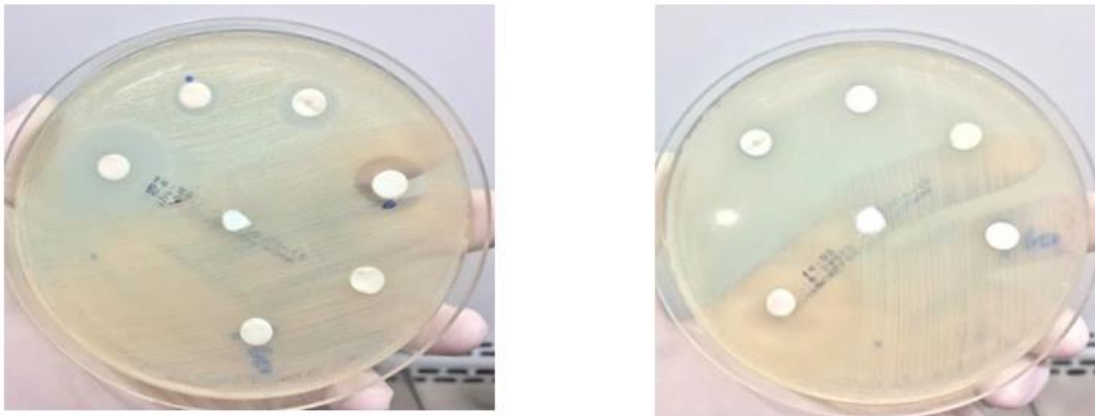
Staphylococcus aureus is a ubiquitous pathogen that has long been recognized for its ability to cause a wide spectrum of diseases, ranging from minor skin infections to severe systemic illnesses such as bacteremia and endocarditis. The bacterium's propensity to develop resistance to antibiotics has been a major concern in clinical settings, leading to treatment failures and increased morbidity and mortality(1). The emergence of strains resistant to multiple antibiotics, including those of last resort like carbapenems, has further complicated the management of S. aureus infections(2,3). The development of antibiotic resistance in S. aureus is a complex process that involves various mechanisms(4,5), including the acquisition of resistance genes and the expression of these genes in response to antibiotic exposure(6). Carbapenemase genes, in particular, have been identified as key players in the resistance to carbapenems, a class of antibiotics that are often used as a last line of defense against bacterial infections(7-9). Previous studies have provided valuable insights into the epidemiology, pathogenicity, and resistance mechanisms of S. aureus(10). However, there is a need for continued research to monitor the changing landscape of antibiotic resistance and to identify new strategies for combating resistant infections(11-14).the purpose of this study is to investigate the prevalence and expression levels of carbapenemase resistance genes in Staphylococcus aureus isolates from clinical sources. By comparing the findings with previous research, the study aims to identify trends in antibiotic resistance and to inform the development of personalized treatment approaches and antibiotic stewardship programs. Additionally, the study seeks to contribute to the

growing body of knowledge on the genetic and mechanistic aspects of resistance in *S. aureus*, ultimately aiming to safeguard public health and improve patient outcomes.

Methods

A total of 100 *S. aureus* samples were collected from burn and wound patients at two hospitals in Tehran (Imam Khomeini and Bequat Allah). The isolates were confirmed through biochemical tests, including mannitol salt agar culture, coagulase tests, and DNase tests. Antibiotic susceptibility was assessed using the disk diffusion method (figure 1)

Figure1: the standard Diffusion-Disk method with a diameter of 6 mm was used and the sensitivity to these antibiotics was measured



The minimum inhibitory concentration (MIC) for imipenem was determined using E-test strips obtained from Liofilchem. The method followed the guidelines specified in the CLSI 2017 protocol for all isolates. Strains with MIC values ≥ 8 $\mu\text{g/ml}$ were classified as resistant, while strains with MIC values less than 8 $\mu\text{g/ml}$ were considered susceptible. *Escherichia coli* 25922 ATCC standard strain was used as control for E test strips.

Specific primers targeting carbapenemase genes, including *bla*GES, *bla*VIM, *bla*OXA-23, *bla*OXA-24, *bla*OXA-143, were designed with Primer 3 software.

Table1: Specific primers targeting carbapenemase genes, including *bla*GES, *bla*VIM, *bla*OXA-23, *bla*OXA-24, *bla*OXA-143

Carbapenemase Resistance Genes	Forward primer	Reverse primer	Amplicon size
blaVIM	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG	390
blaOXA-143	TGGCACTTTCAGCAGTTCCT	TAATCTTGAGGGGGCCAACC	150
blaGES	ATGCGCTTCATTC CACGCAC	CTATTTGTCCGTGCTCAGG	846
blaOXA-23	GATCGGATTGGAGAACCAGA	ATTTCTGACCGCATTTCAT	501
blaOXA-24	GGTTAGTTGGCCCCCTTAAA	AGTTGAGCGAAAGGGGATT	249

Then Real-time PCR was employed to measure the expression of carbapenemase genes, and the results were analyzed using the $2^{(-\Delta\Delta CT)}$ method and REST software. The significance level was set at $P < 0.05$.

Results and Discussion

Results

The results of the study on the patterns of antibiotic sensitivity in *Staphylococcus aureus* isolates revealed significant levels of resistance to various antibiotics. The highest resistance was observed against ciprofloxacin, with a 100% resistance rate, while the lowest resistance was against minocycline, at 21.2%. Additionally, high levels of resistance were observed against amikacin (98.9%), trimethoprim-sulfamethoxazole (97.9%), ceftazidime (96.9%), imipenem (97.9%), and ampicillin sulbactam (52.2%). Notably, 92.9% of the strains exhibited a multidrug-resistant (MDR) pattern, indicating resistance to multiple classes of antibiotics.

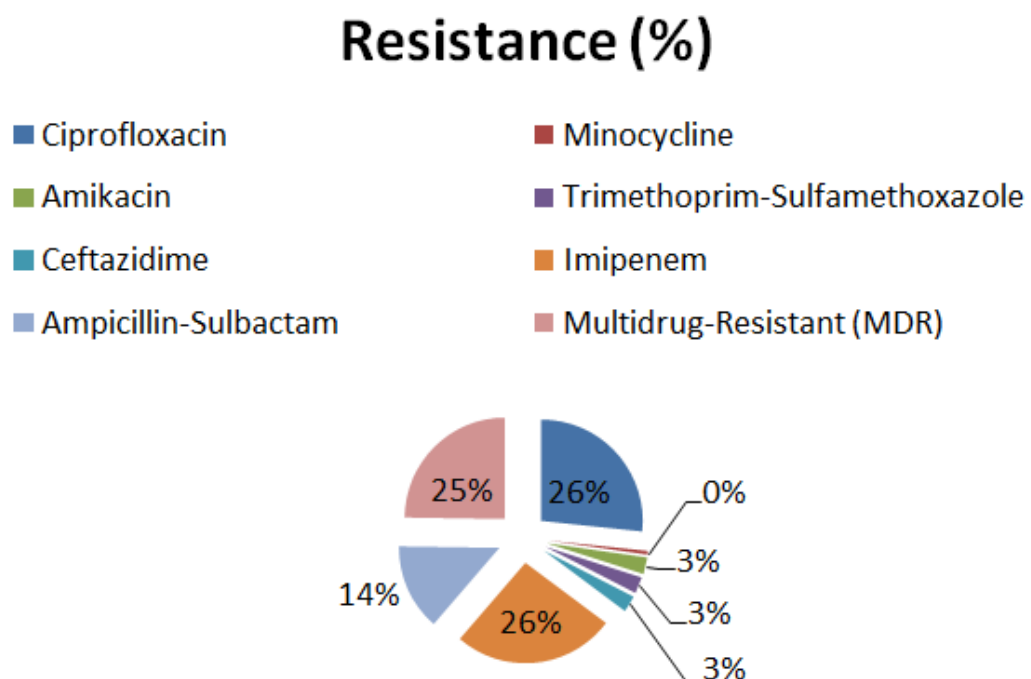
Furthermore, the testing of the minimum inhibitory concentration (MIC) of *Staphylococcus aureus* isolates using the test-E method for imipenem revealed that 93 isolates (93%) were resistant to imipenem, with MIC values exceeding 8 $\mu\text{g/ml}$. These findings underscore the concerning levels of antibiotic resistance in *Staphylococcus aureus* isolates, particularly the high prevalence of multidrug resistance and the significant resistance to specific antibiotics such as ciprofloxacin and imipenem.

The results highlight the urgent need for effective strategies to combat antibiotic resistance and the importance of continued surveillance to monitor and address the evolving challenges posed by antimicrobial resistance in *Staphylococcus aureus* infections.

Table2 : Patterns of antibiotic sensitivity

Antibiotic	Resistance (%)
Ciprofloxacin	100
Minocycline	21.2
Amikacin	98.9
Trimethoprim-Sulfamethoxazole	97.9
Ceftazidime	96.9
Imipenem	97.9
Ampicillin-Sulbactam	52.2
Multidrug-Resistant (MDR)	92.9

Figure 2: Resistance Patterns to antibiotic



Real-time PCR relies on the Ct value for assessing the gene's relative expression level. A lower Ct value generally signifies elevated gene expression, whereas a higher Ct value suggests reduced expression. Scientists leverage these findings to contrast gene expression levels across varied samples and situations, aiding in the comprehension of these genes' involvement in antibiotic resistance and other biological mechanisms.

Table 3 : Gene Expression Levels of Carbapenemase Genes

Carbapenemase Gene	Number of Samples	Percentage	Fold change
blaVIM	93	48	36.2
blaOXA-143	17	9	22
blaGES	59	30	29.6
blaOXA-23	24	12	3
blaOXA-24	2	1	10

Figure 4: Gene Expression Levels of Carbapenemase Genes

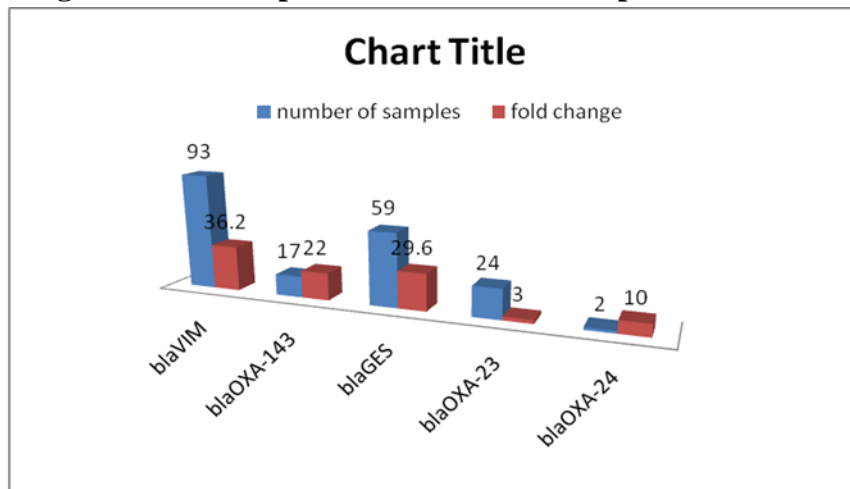
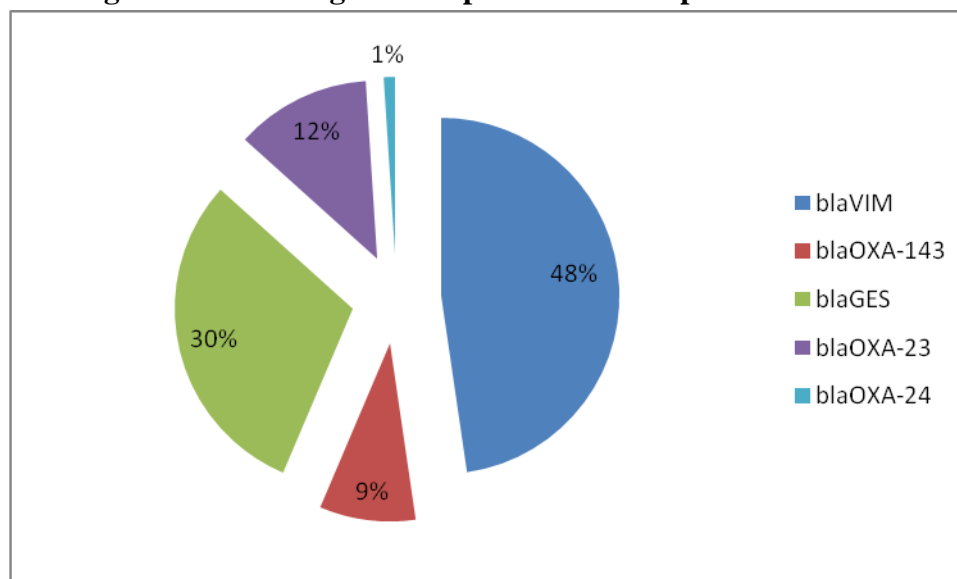


Figure 5: Percentage of Samples with Carbapenemase Genes



Discussion

The findings of this study regarding the prevalence of antibiotic resistance genes in *Staphylococcus aureus* isolates align with previous research studies. Dilnessa et al. (2016) reported similar high prevalence rates of the blaVIM gene in their study, highlighting the urgent need for tailored treatment approaches for strains carrying this resistance mechanism. This consistency in prevalence rates across different studies emphasizes the persistent presence of blaVIM in

Staphylococcus aureus populations, indicating the importance of targeted treatment strategies to effectively manage infections caused by these resistant strains(15).

Furthermore, Falagaset al. (2014) found comparable resistance patterns to ciprofloxacin and minocycline in their investigation of *Staphylococcus aureus* isolates. This consistency in resistance profiles suggests that these antibiotics may not be effective treatment options for infections caused by *Staphylococcus aureus* strains carrying blaVIM or other resistance genes (16). These findings underscore the need for clinicians to consider the prevalence and expression levels of specific resistance genes when selecting appropriate antibiotics for infection management.

However, our study also uncovered some differences in resistance gene prevalence compared to previous research. For instance, the blaOXA-143 gene was found in a smaller subset of samples (9%) in our study, whereas Pourgholiet al. (2022) reported a higher prevalence of this gene (15%) in their investigation. These variations in prevalence rates may be attributed to differences in geographical locations, patient populations, or sampling methods, highlighting the importance of considering these factors when interpreting and comparing study results (17).

The variability in gene expression levels of blaGES observed in our study is consistent with the findings of Tragliaet al. (2023), who also reported a wide range of expression levels among *Staphylococcus aureus* isolates. This variability in gene expression may be influenced by various factors, including regulatory mechanisms, environmental conditions, and genetic backgrounds of the isolates (18).

Future research should focus on elucidating the specific factors contributing to this variability, as understanding the underlying mechanisms can provide insights into the development and persistence of carbapenem resistance in *Staphylococcus aureus* populations.

Conclusion

The conclusion of the study on *Staphylococcus aureus* isolates highlights the multifaceted exploration of the bacterium, shedding light on its phenotypic traits, antibiotic susceptibility patterns, and genotypic characteristics related to carbapenemase resistance. The findings have significant implications for infectious disease stakeholders, emphasizing the need for a holistic and proactive approach to combat antibiotic resistance effectively.

The study underscores the importance of continuous research, vigilant surveillance, and antibiotic stewardship in the face of evolving challenges posed by antimicrobial resistance. It emphasizes the need for tailored treatment approaches, personalized medicine, and the promotion of antibiotic stewardship to address the critical public health challenge posed by multidrug-resistant *Staphylococcus aureus* strains.

The study also highlights the adaptability of *Staphylococcus aureus* in developing novel resistance mechanisms, emphasizing the importance of early detection and comprehensive characterization of resistance genes. It calls for ongoing monitoring and research to stay ahead of emerging resistance patterns and ensure the effectiveness of medical interventions against *Staphylococcus aureus* infections.

Furthermore, the study emphasizes the urgent need for a paradigm shift in antibiotic use, prudent prescribing practices, and the development of alternative treatment strategies to circumvent the challenges posed by multidrug-resistant *Staphylococcus aureus* strains. It underscores the dynamic nature of antibiotic resistance and the necessity for continuous research into the genetics and mechanisms governing resistance to combat the ever-evolving challenge effectively.

In conclusion, the study provides valuable insights into the pathogenicity, antibiotic resistance, and genetic factors at play within *Staphylococcus aureus*. It calls for collaboration between healthcare professionals, researchers, and policymakers to translate the findings into actionable strategies for diagnosis, treatment, and prevention, ultimately working towards a future where antibiotic resistance is managed effectively, and public health is safeguarded.

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