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Investigation on marine *Staphylococcus* spp. isolated from the Sinop coastal areas, the Black Sea in Türkiye serves as a reservoir for antibiotic resistance genes

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ABSTRACT

Epidemiological surveillance of the *Staphylococci* genus, which harbours strains with high morbidity and mortality rates, is a crucial step in detecting and preventing diseases and disease agents. This study aimed to isolate, characterise, and screen some antibiotic resistance genes of possible *Staphylococcus* spp. strains from seawater samples taken from three points in Sinop, which is suitable for swimming from almost every point. Classical microbiological techniques were used for the isolation and possible identification of the strains. A fragment of the 16S rRNA gene region (216 bp) was amplified and analysed by the SSCP technique to determine their diversity among themselves. For antibiotic resistance genes, both classical PCR and multiplex PCR techniques were used. As a result, 29 probable *Staphylococcus* spp. strains were isolated, and according to SSCP analysis, it was determined that the strains had a similarity rate of 50% or more among themselves and within the scope of different stations. In addition, *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM*, and *blaZ* resistance genes of the strains were observed as 8 (27.5%), 3 (10.3%), 2 (6.8%), 2 (6.8%), 14 (48.2%), 27 (93.1%) and 29 (100%), respectively. Furthermore, *mecA* was positively correlated with *ermB* and *ermB* was positively correlated with *ermC* at the $p < 0.05$ significance level. In comparison, *ermB* was negatively correlated with t*etM* at the $p < 0.05$ significance level. In conclusion, the presence of *Staphylococcus* spp. strains, which are reservoirs of antibiotic resistance genes and have the potential to transfer these genes to other bacteria through gene transfer, have been shown in this study to be prevalent in marine environments, where they can be easily transmitted. The importance of taking precautions has been emphasised.

Keywords: *Staphylococcus* spp., SSCP, *mecA*, Seawater, Antibiotic resistance genes

Introduction

Due to the mortality and financial losses caused by infections, antimicrobial resistance (AMR) is becoming a significant issue in communities. An analysis conducted in 2019 estimated that antibiotic resistance resulted in the deaths of 1.27 million individuals in Europe alone (Brauge et al., 2024). The prevalence of such infections is a significant concern associated with this condition. Marine water has been reported to be one of the most harmful settings for them. Millions of cutaneous, acute respiratory, and gastrointestinal disorders are expected to occur yearly as a result of microbial pollution in marine water habitats (Goodwin et al., 2012). Due to their high mortality rates and multidrug resistance (MDR), the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) have been identified as the most significant risk factors for these illnesses. The fact that the ESKAPE group demonstrates diverse antibiotic resistance and transfers its resistance genes to other bacteria through horizontal gene transfer is one of the most critical considerations (Ackers-Johnson et al., 2024).

A range of Staphylococcus species currently produces serious infections. Humans are hosts to 17 of the 47 species and 24 subspecies that make up the genus *Staphylococcus*. The three that are most hazardous to humans are *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Thirty percent of nosocomial infections and 1-5% of community infections have been ascribed to *S. aureus* (Alabbosh et al., 2023).

The emergence of antibiotic-resistant strains of *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), is a global concern and cause for concern when this ESKAPE category is evaluated. The World Health Organisation's (WHO) categorisation of MRSA as a high-priority disease draws increased attention due to its resistance to numerous medications (Cui et al., 2024). According to a past study on *S. aureus* in coastal locations, swimmers can contract the bacterium from saltwater by shedding it through their skin, nose, and respiratory system. *S. aureus* has occasionally been identified in high quantities in both the water and the sand on recreational beaches; this can be directly linked to the number of swimmers and the quantity of people utilising the beach (Akanbi et al., 2017).

Research has been used to track and document the emergence of antibiotic resistance in *S. aureus* strains throughout time. These comprise multidrug-resistant *S. aureus* (resistant to penicillin-G, chloramphenicol, tetracycline, and erythromycin) in the late 1950s and penicillin-G-resistant *S. aureus* that developed penicillinase (penicillin-hydrolysis enzyme) in the mid-1940s. Methicillin, a β-lactam antibiotic that works against penicillin-resistant *S. aureus*, was initially sold in the 1960s, but strains of MRSA were found a year later. All β-lactams were unsuccessful against this MRSA (Contractor et al., 2024).

Methicillin resistance is imparted by the production of penicillin-binding protein 2a (PBP2a), which is carried by *mecA* on staphylococcal cassette chromosome *mec* (SCC*mec*). This mobile genetic element carries cassette chromosome recombinase genes. Even though SCC*mec* transfer is unusual in *S. aureus*, methicillin-sensitive *S. aureus* (MSSA) strains convert into MRSA after getting *mecA* embedded in SCC*mec*; all MRSA clones seem to include specific kinds of SCC*mec* (Takahashi et al., 2024; Vittorakis et al., 2024; Wan et al., 2025). In addition to *mecA*, resistance genes against tetracyclines and aminoglycosides can also be transferred in the SCC*mec* cassette, one of the 14 different cassettes found in staphylococci. Tetracycline decreases the creation of proteins by interacting with 16S rRNA genes. The *tetK* and *tetL* genes encode efflux pumps that actively remove tetracyclines from the cells, which is the primary mechanism of bacterial resistance to these antibiotics. The alternate technique utilizes ribosome protection proteins, which are generated by the *tetO* and *tetM* genes. These proteins either break down tetracyclines or inhibit them from binding by modifying the ribosome's structure (Szemraj et al., 2025). MRSA strains can demonstrate multidrug resistance, or resistance to antibiotics from various classes, including aminoglycosides, macrolides, tetracyclines, and fluoroquinolones, in addition to their innate resistance to β-lactam antibiotics (Michalik et al., 2025).

S. aureus has acquired diverse resistance mechanisms to β-lactam antibiotics, primarily associated with the *blaZ* and *mecA* genes. *mecA* is not effectively inhibited by β-lactams and permits bacterial cell wall cross-linking even in the presence of antibiotics. The *blaZ* gene produces the β-lactamase enzyme, which breaks down susceptible β-lactam antibiotics (Hnini et al., 2024). *S. aureus* can also have resistance genes such as the erythromycin resistance genes *erm*(B) and *erm*(C), the chloramphenicol resistance gene *cat*, and the lincomycin resistance gene *lnu*(A), in addition to the ones mentioned above (Cui et al., 2024).

This work aimed to characterise *Staphylococcus* spp. strains isolated from seawater samples in Sinop, Turkey, and to screen them for the antibiotic resistance genes *mecA*, *blaZ*, *tetK*, *tetM*, *ermA*, *ermB*, and *ermC*.

Materials and Methods

Collection of Samples

Water samples were obtained under aseptic conditions on June 4, June 10, June 26, July 9, and July 16, 2024, from Karakum (coordinates: 42°00'56"N 35°11'07"E), Taşocağı (coordinates: 42°01'03"N 35°10'10" E), and Kumkapı (coordinates: 42°01'33"N 35°08'21" E) in the center of Sinop, Turkey (Figure 1). Seawater samples were obtained in 500 mL sterile bottles from a few millimetres beneath the surface, ensuring no exposure to air, and transported to the laboratory in a cold chain bag within two hours for immediate examination.

Isolation of Staphylococcus spp. *Strains*

Fifteen different water samples were inoculated on Mannitol Salt Agar (MSA, Merck, Germany) and incubated at 30°C for 24 hours immediately after being brought to the laboratory to isolate possible *Staphylococcus* strains. At the end of the period, yellow-colored colonies were selected and grown in Nutrient Broth (Merck), and pure cultures were prepared (Faria et al., 2009). Isolates selected as single colonies were stored at -80°C in 20% glycerol stock. Gram staining, catalase, and oxidase tests were performed for the identification of probable *Staphylococcus* strains and further tests were performed with 29 strains selected according to these tests.

Genomic DNA isolation and 16S rRNA SSCP analysis

Genomic DNA isolation of the 29 strains was performed according to Sambrook et al. (1989) and stored at -20°C until analysis.

For single-stranded conformation polymorphism (SSCP) analysis performed with primers P11P and P13P (Table 1) selected to amplify the 216 bp fragment expressed as V6 in the 16S rRNA gene region, the method was as described in a previous study conducted in the same laboratory (Avsar et al., 2017). Briefly, the amplification conditions for SSCP included an initial denaturation at 95°C for 4 minutes. This was followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes. Two microliters of PCR product were added to 10 µL of denaturation solution (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanole and 10 mM NaOH). The mixture was heated to 95°C for 5 minutes and then immediately cooled on ice. The mixture was analysed by non-denaturing polyacrylamide gel electrophoresis (39:1 acrylamide: bisacrylamide) in 0.5 x TBE (16 x 18 cm) for 27 h at 18°C at a constant power of 5 mA on a Hoefer electrophoresis system (Hoefer Inc., Holliston, MA, USA). The gel was silver nitrate-stained and photographed. After that, a dendrogram based on UPGMA was produced using GelJ (Heras et al., 2015).

Figure 1. Map of the stations where samples were taken (Google map 2025). Karakum, Taşocağı, and Kumkapı stations are the places where people swim during the sampling period

Table 1. All primer sequences and sizes used in the study

Gene		The primer sequences (5'- 3')	Size of amplified product (bp)
P11P P13P	Forward Revers	**GAGGAAGGTGGGGATGACGT** **AGGCCCGGGAACGTATTCAC**	**216**
mecA	Forward Revers	**AAAATCGATGGTAAAGGTTGGC** **AGTTCTGCAGTACCGGATTTGC**	**533**
ermA	Forward Revers	**AAGCGGTAAACCCCTCTGA** **TTCGCAAATCCCTTCTCAAC**	**190**
ermB	Forward Revers	**CTATCTGATTGTTGAAGAAGGATT** **GTTTACTCTTGGTTTAGGATGAAA**	**142**
ermC	Forward Revers	**AATCGTCAATTCCTGCATGT** **TAATCGTGGAATACGGGTTTG**	**299**
tetK	Forward Revers	**GTAGCGACAATAGGTAATAGT** **GTAGTGACAATAAACCTCCTA**	**360**
tetM	Forward Revers	**AGTGGAGCGATTACAGAA** **CATATGTCCTGGCGTGTCTA**	**158**
blaZ	Forward Revers	**ACTTCAACACCTGCTGCTTTC** **TGACCACTTTTATCAGCAACC**	**173**

Detection of Antibiotic Resistance Genes

The primer base sequences of *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ*, preferred to detect antibiotic resistance genes, are given in Table 1.

Amplification of the *mecA* gene was performed using *mecA*-F and *mecA*-R primers, and a PCR product of 533 bp was obtained. PCR was performed in a volume of 25 µL with PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 µL 10 µM dNTPs, 0.4 µL Taq polymerase (DreamTaq) and 0.125 µM concentration of each primer. For amplification, initial denaturation was performed using 40 cycles of amplification at 94°C for 3 min, 94°C for 30 s, 55°C for 30 s and 72°C for 30 s; this reaction was followed by an additional extension of 5 min at 72°C (Lee, 2003). Sequences of other primers preferred for antibiotic resistance were taken from Duran et al. (2012). In addition, multiplex PCR was performed using tet and erm (annealing was performed at 55 °C for the multiplex PCR) primer sets, except for blaZ (annealing was performed at 54°C for the PCR) in the screening of these gene regions (Duran et al., 2012).

Statistical Analyses

The Pearson correlation was utilised to examine the association between antibiotic resistance genes using Past 4.03 statistical software; *p*-values lower than 0.05 were considered statistically significant.

Results and Discussion

According to Gram staining and catalase and oxidase tests, a total of 29 putative *Staphylococcus* strains were identified. Cocci morphology (more like grape clusters) is known to be specific for these bacteria, which makes their differentiation by classical microbiological tests easier. Out of the 29 strains among these strains, 11 were isolated from Karakum, 10 from Taşocağı, and 8 from Kumkapı (Table 2).

A 216 bp fragment of 16S rRNA was analysed using SSCP to assess the similarity among 29 isolated *Staphylococcus* spp. Bacteria, resulting in the construction of a UPGMA dendrogram (Figure 2). Consequently, it was established that the strains exhibited 50% or greater similarity to one another. Upon evaluation of the strains isolated from three distinct stations, it was ascertained that they exhibited diversity and were categorised into various clusters. Although techniques such as SSCP are still employed for the identification and diversity of potentially harmful bacterial groups such as *Staphylococcus*, faster methods have been studied in recent years. Zhao et al. (2024) indicated that the HiFi-loop-mediated isothermal amplification (LAMP) method created for expedited identification of MRSA and MSSA strains was exceptionally effective. In another study, Zhang et al. (2025) achieved an ultra-simple self-assembly of gold nanoparticles (AuNPs) using Nisin. They successfully applied lateral flow immunoassay

(LFIA) by utilising Nisin as a recognition element in combination with a smartphone to construct a dual-read detection sensor for the rapid detection of MRSA. In another work, Yang et al. (2025) developed an AI-driven colour-coded multiplex hydrogel LAMP approach that has promising possibilities for the digital quantification of antibiotic-resistant bacteria (such as *Escherichia coli* and MRSA) in the food market. In another investigation, Li et al. (2025) used the SELEX (the Systematic Evolution of Ligands by Exponential Enrichment) approach for optical detection of *S. aureus* in fewer than 9 hours. Here, they completed a specific identification based on a magnetic bead target enrichment and rolling circle amplification approach to enhance sensitivity, utilising a dual aptamer combined recognition target strategy. These approaches highlight only a few of them, clearly indicating that the timely diagnosis of *Staphylococcus* species, especially in food and environmental samples, is vital for preventing possible infections.

The antibiotic resistance genes of the 29 tested *Staphylococcus* spp. strains were screened and displayed in Table 2 and Figure 3A-B. Accordingly, *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ* resistance genes were found as 8 (27.5%), 3 (10.3%), 2 (6.8%), 2 (6.8%), 14 (48.2%), 27 (93.1%) and 29 (100%), respectively. It is notable that *mecA*, an indicator of methicillin-resistant *Staphylococcus* strains, was found in 8 strains from three distinct stations. In addition, it is interesting that all strains possessed the *blaZ* resistance gene and the *tetM* resistance gene was identified in all but two of them.

In the context of this work, although we have focused on separating Staphylococcus strains from seawater, we can observe that this group of bacteria is widespread and strongly represented. The importance of this group becomes clear when considering the studies completed in the last few years alone. Some of these studies are as follows: Oshamika et al. (2024) examined antibiograms and harmful genes of *S. aureus* in asthmatic children. Ackers-Johnson et al. (2024) isolated *Staphylococcus* spp. from door knobs and push panels from a hospital in Liverpool and looked at their antibiotic resistance. Agredo-Campos et al. (2025) isolated *S. aureus* from milk tanks and studied their characterisation, antibiograms, pathogenicity and antibiotic resistance. Desire et al. (2024) isolated MRSA from smoked fish and analysed the behavioural and genotypic characterisation of the *mecA* genes. Dewi et al. (2024) studied the antibiograms, *mecA* and PVL gene presence of multidrug-resistant *Staphylococcus* isolated from an international airport. Olivo et al. (2024) isolated methicillin-resistant *Staphylococcus* spp. from individuals in contact with sick horses at a veterinary hospital and investigated their characteristics and the presence of the *mecA* gene. These research studies, conducted in various fields over the last one to two years, highlight the importance and prevalence of the genus *Staphylococcus*.

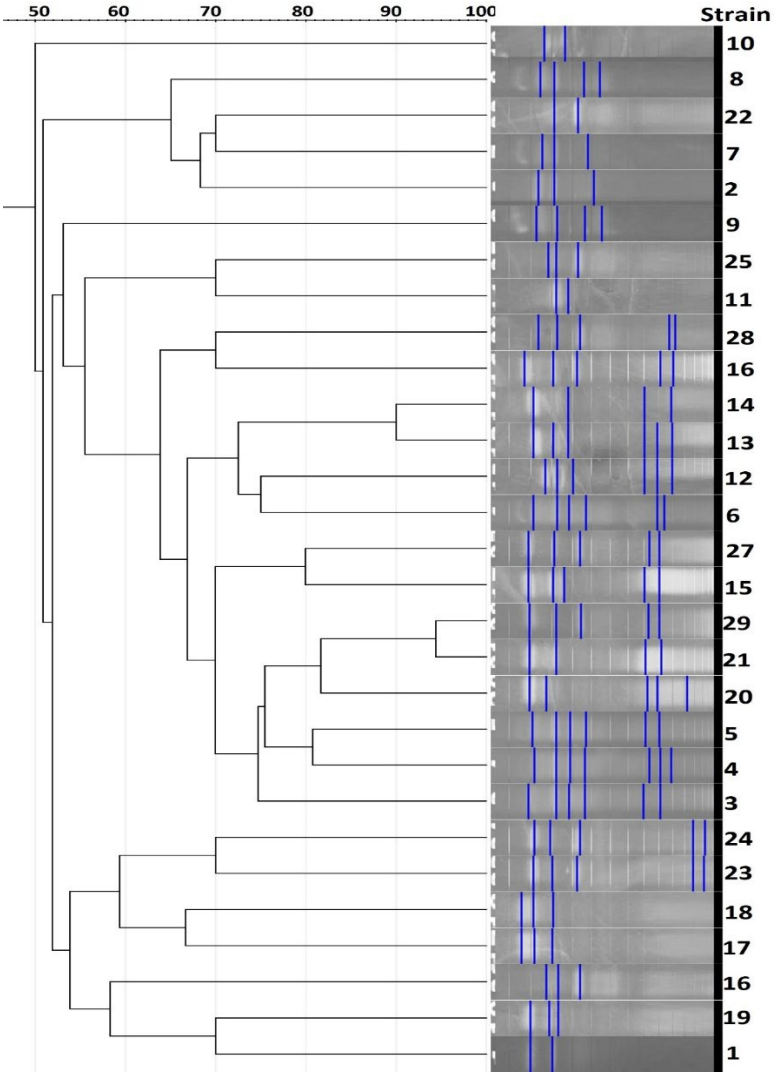

Figure 2. Phylogenetic tree constructed based on UPGMA analysis

In addition to these studies, there have been many studies in which *Staphylococcus* species have been isolated from seawater samples, characterised, and their resistance to antibiotics and resistance genes, including *mecA*, have been investigated (Soge et al., 2006; Harekeh et al., 2006; Goodwin et al., 2012; Akanbi et al., 2017; Gerken et al., 2021; Brauge et al., 2024). The fact that our work is in many ways comparable to previous studies on staphylococci isolated from seawater demonstrates the inevitability of protection against possibly pathogenic *Staphylococcus* for seawater.

In addition, the Pearson's correlation between different antibiotic resistance genes was studied and displayed in Figure 4.

Accordingly, the *mecA* had a significant impact on the *erm* resistance genes, especially with the *ermB* at the $p < 0.05$ level. On the other hand, the *mecA* demonstrated a very modest level of negative connection with both the *tet* resistance genes. In addition, the *ermB* was favorably linked with the *ermC* (+ 0.46) and negatively connected with the *tetM* (- 0.46) at a $p < 0.05$ significance level. Alkuraythi et al. (2024) evaluated the correlation coefficient between *mecA*, *tet* and *ermC* resistance genes in *S. aureus* strains and found that their results were parallel to our findings. In another work, Fontana et al. (2021) examined the correlation between *ermA*, *B*, *C* and *tetK* resistance genes in coagulase-negative *Staphylococcus* isolates obtained by them. While the connection between *ermA*, *ermB*, and *tetK* was the same as our findings, a difference was observed in the correlation between *ermC* and *tetK*, albeit extremely modest.

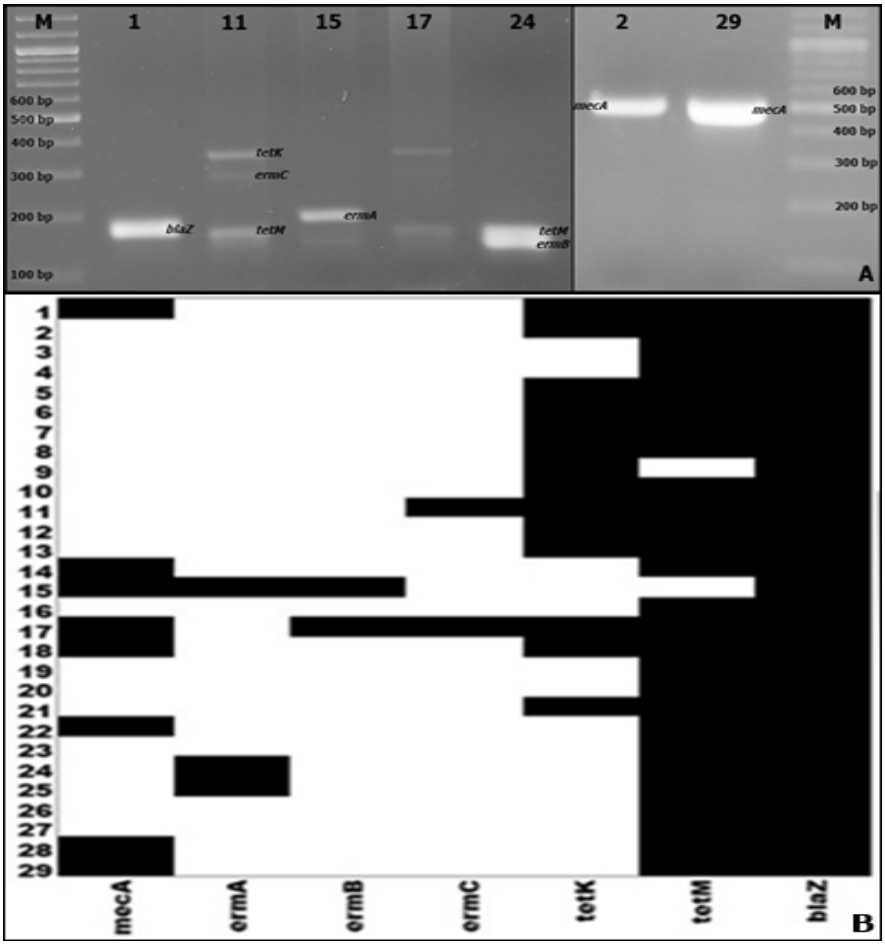

Figure 3. Antibiotic resistance genes screening results; (A) Agarose gel images of tested antibiotic resistance genes. M (Marker - 1000 bp GeneRuler); *blaZ* (173 bp) for strain 1; *tetK* (360 bp), *ermC* (299 bp) and *tetM* (158 bp) for strain 11; *ermA* (190 bp) for strain 15; *blaZ* and *tetM* for strain 17; *tetM* and *ermB* (142 bp) for strain 24; *mecA* (533 bp) for strains 2 and 29. (B) Results for all the strains show that the black part opposite the gene is positive for the strain, while the white part is negative. This type of graph was generated from the Past 4.03 program

Table 2. Stations where strains were isolated and the antibiotic resistance gene screening results

Strain	Location	*mecA*	*ermA*	*ermB*	*ermC*	*tetK*	*tetM*	*blaZ*
1	Karakum	+	-	-	-	+	+	+
2	Karakum	-	-	-	-	+	+	+
3	Karakum	-	-	-	-	-	+	+
4	Karakum	-	-	-	-	-	+	+
5	Karakum	-	-	-	-	+	+	+
6	Karakum	-	-	-	-	+	+	+
7	Karakum	-	-	-	-	+	+	+
8	Karakum	-	-	-	-	+	+	+
9	Karakum	-	-	-	-	+	-	+
10	Karakum	-	-	-	-	+	+	+
11	Karakum	-	-	-	+	+	+	+
12	Taşocağı	-	-	-	-	+	+	+
13	Taşocağı	-	-	-	-	+	+	+
14	Taşocağı	+	-	-	-	-	+	+
15	Taşocağı	+	+	+	-	-	-	+
16	Taşocağı	-	-	-	-	-	+	+
17	Taşocağı	+	-	+	+	+	+	+
18	Taşocağı	+	-	-	-	+	+	+
19	Taşocağı	-	-	-	-	-	+	+
20	Taşocağı	-	-	-	-	-	+	+
21	Taşocağı	-	-	-	-	+	+	+
22	Kumkapı	+	-	-	-	-	+	+
23	Kumkapı	-	-	-	-	-	+	+
24	Kumkapı	-	+	-	-	-	+	+
25	Kumkapı	-	+	-	-	-	+	+
26	Kumkapı	-	-	-	-	-	+	+
27	Kumkapı	-	-	-	-	-	+	+
28	Kumkapı	+	-	-	-	-	+	+
29	Kumkapı	+	-	-	-	-	+	+

(+) positive result; (−) negative result

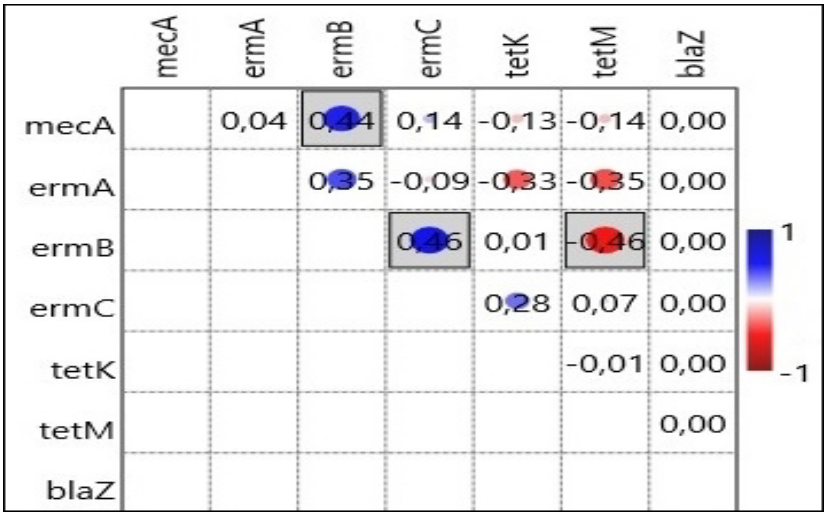

Figure 4. Pearson correlation results between antibiotic resistance genes. Boxes indicate $p < 0.05$ significance value.

Conclusion

Within the framework of this investigation, *Staphylococcus* spp. strains were isolated from samples collected at three different swimming locations in Sinop Province during the period when people typically swim in the sea, and their diversity was examined. In addition, specific antibiotic resistance genes were screened, and some strains were identified as harbouring various antibiotic resistance genes, primarily *mecA* (27.5%). It is significant because they are both reserved for resistance genes and are likely to convey these genes, possibly by horizontal gene transfer. The presence of such bacterial groups in the seas, which are employed primarily for leisure purposes and most significantly for fishing, should lead to severe measures. Otherwise, the impact of the transmission of the genus *Staphylococcus*, which has a high morbidity and mortality rate among certain of its species, which are frequently recorded in the literature, through seawater may be catastrophic.

Compliance with Ethical Standards

Conflict of interest: The author declares no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: Data will be made available on request from the author.

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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