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RESEARCH ARTICLE

Assessment of Antibiotic-Resistant Bacteria in Recreational Coastal Waters of Çanakkale, Türkiye

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Abstract: This study investigates the prevalence of antibiotic-resistant bacteria in recreational coastal waters along the Çanakkale Strait (Türkiye), a region experiencing increasing anthropogenic pressure from urbanization, maritime activity, and seasonal tourism. A total of 211 bacterial isolates were obtained from surface seawater samples collected at 14 different recreational coastal sites during the summer season of 2024. These isolates were identified using the VITEK® 2 Compact system and tested against 15 antibiotics via the Kirby-Bauer disk diffusion method, following CLSI (2018) guidelines. The results revealed alarmingly high resistance rates, with vancomycin (92.89%), kanamycin (81.04%), and sulphonamides (64.45%) being the most affected. The Multiple Antibiotic Resistance (MAR) index ranged from 0.14 to 0.71, with over 95% of isolates scoring ≥ 0.2 , indicating exposure to high-risk environments. *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus intermedius* and *Bacillus cereus* were among the most frequently isolated multidrug-resistant species. Comparative analysis with other marine studies from Türkiye confirms that Çanakkale's coastal waters are significantly impacted by antibiotic contamination. The findings highlight the urgent need for regular surveillance and enhanced wastewater treatment strategies to mitigate the spread of resistance genes in coastal ecosystems. This study contributes essential baseline data to the national inventory on marine antibiotic resistance and underscores the public health risks posed by recreational waterborne exposure to resistant bacteria.

Anahtar kelimeler:

Antibiyotik dirençliliği
Heterotrofik bakteri
Deniz suyu
Çanakkale Boğazı

Çanakkale Rekreatiyonel Kıyı Sularında Antibiyotik Dirençli Bakterilerin İncelenmesi

Öz: Bu çalışma, artan kentleşme, denizcilik faaliyetleri ve mevsimsel turizm nedeniyle insan kaynaklı baskı altındaki Türkiye'nin Çanakkale Boğazı kıyılarındaki rekreatiyonel sulara antibiyotik dirençli bakterilerin yaygınlığını araştırmaktadır. 2024 yaz döneminde, 14 farklı rekreatiyonel kıyı noktasından alınan yüzey deniz suyu örneklerinden toplam 211 bakteri izolatu elde edilmiştir. Bu izolatlar, VITEK® 2 Compact sistemi kullanılarak tanımlanmış ve CLSI (2018) standartlarına uygun şekilde Kirby-Bauer disk difüzyon yöntemi ile 15 farklı antibiyotiğe karşı test edilmiştir. Sonuçlar, en yüksek direnç oranlarının sırasıyla vankomisin (%92,89), kanamisin (%81,04) ve sülfonamidler grubu (%64,45) antibiyotiklere karşı geliştiğini göstermiştir. Multiple Antibiotic Resistance (MAR) indeks değerleri 0.14 ile 0.71 arasında değişmiş; izolatların %95'inden fazlası ≥ 0.2 değerinde bulunarak yüksek riskli çevresel maruziyeti işaret etmiştir. En sık izole edilen çoklu dirençli bakteri türleri arasında *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus intermedius* ve *Bacillus cereus* yer almıştır. Türkiye'de yapılan diğer denizel çalışmalarla karşılaştırmalı analizler, Çanakkale kıyı sularının antibiyotik kontaminasyonundan önemli ölçüde etkilendiğini doğrulamaktadır. Elde edilen bulgular, direnç genlerinin kıyı ekosistemlerinde yayılımını önlemek amacıyla düzenli çevresel izleme ve atık su arıtma stratejilerinin güçlendirilmesi gerekliliğini vurgulamaktadır. Bu çalışma, denizel antibiyotik dirençliliği konusunda ulusal veri tabanı için temel veri sağlamakta ve rekreatiyonel su yoluyla dirençli bakterilere maruz kalmanın halk sağlığı açısından oluşturduğu risklerin altını çizmektedir.

Introduction

Antibiotics are widely used pharmaceutical agents for treating infections in both human and veterinary medicine. However, the excessive and uncontrolled use of these

substances has led to the emergence of antibiotic-resistant bacterial populations in various environments, including aquatic systems. A significant portion of administered

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antibiotics is not fully metabolized and is introduced into the environment through domestic wastewater, hospital effluents, and agricultural runoff, eventually reaching coastal and marine waters (Kümmerer, 2009; Larsson, 2014).

The presence of antibiotic-resistant bacteria (ARB) in natural water bodies is not only an ecological concern but also a serious threat to public health. These bacteria can act as reservoirs for antibiotic resistance genes (ARGs), which can be horizontally transferred to pathogenic species, potentially leading to infections that are more difficult to treat (Baquero et al., 2008; Marti et al., 2014).

Numerous studies in Türkiye have demonstrated high rates of antibiotic resistance in marine bacteria. For instance, Matyar et al. (2008) reported ampicillin resistance in 94.4% of bacterial isolates from sediment samples in İskenderun Bay. Similarly, in Güllük Bay, bacteria isolated from seawater and sediment exhibited near-complete resistance to sulfonamides, rifampicin, and oxytetracycline (Altuğ et al., 2020).

The Dardanelles Strait (Çanakkale Boğazı) is a highly dynamic water body with significant ship traffic and coastal recreational activity, particularly during summer months. Despite its ecological and public health relevance, there is a lack of systematic research assessing antibiotic

resistance levels in bacteria isolated from recreational coastal waters in this region.

The aim of this study is to investigate the antibiotic resistance profiles of heterotrophic bacteria isolated from seawater samples collected at popular recreational swimming areas along the Çanakkale coast. The findings are intended to contribute to microbial water quality assessment and raise awareness of the potential public health risks associated with antibiotic-resistant bacteria in coastal environments.

Material and Methods

This study was conducted using seawater samples collected from multiple recreational coastal sites along the Dardanelles Strait (Çanakkale, Türkiye), where public swimming is common (Figure 1). A total of 14 sampling stations were selected based on proximity to human settlements, degree of coastal use, and potential pollution sources (Table 1). Sampling took place during the summer season of 2024, covering the months of June, July, and August.

At each site, 500 mL of surface seawater was collected using sterile glass bottles. Samples were transported to the laboratory under cold chain conditions ($\sim 4^{\circ}\text{C}$) and processed within six hours of collection.

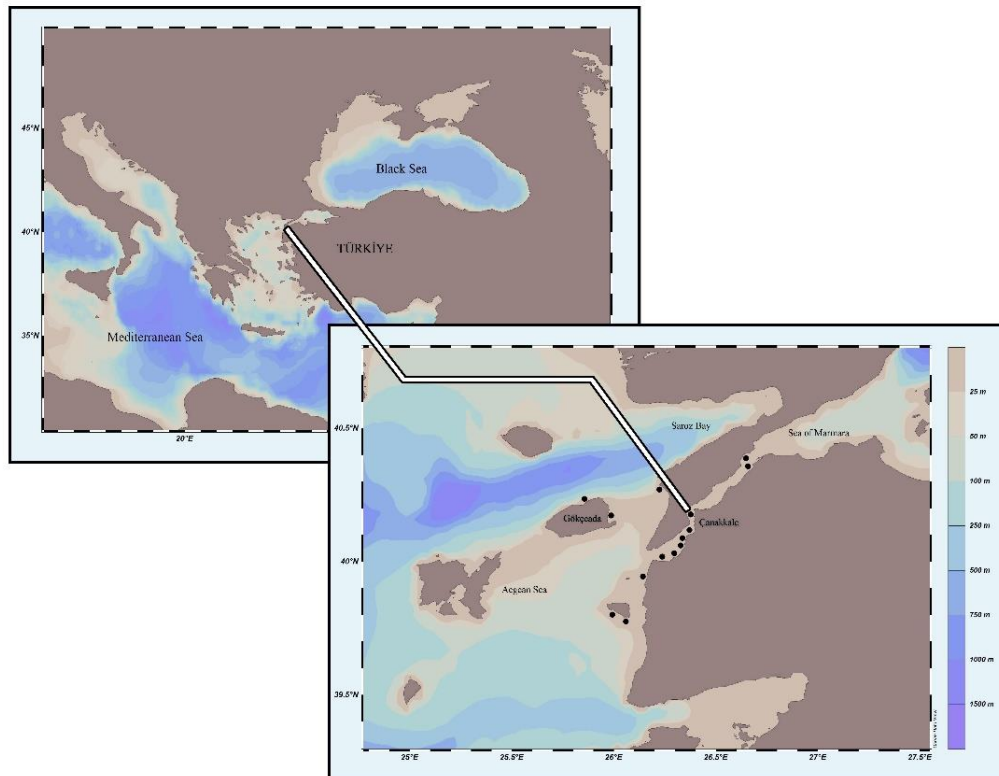


Figure 1. The sampling stations in the Çanakkale Strait

Table 1. Names and coordinates of the stations where sea water samples were collected

Station No	District	Sampling Site Name	Latitude - Longitude
1	Ayvacic	Assos Bathing Water Site	39°29'070"N - 26°20'210"E
2	Ayvacic	Küçükkuyu Inner Harbor Recreational Coastal Water	39°32'680"N - 26°36'380"E
3	Biga	Karabiga Public Beach	40°23'774"N - 27°18'865"E
4	Bozcaada	Ayazma Public Beach	39°48'636"N - 26°00'600"E
5	Bozcaada	Akvaryum Bay Bathing Water Site	39°48'203"N - 26°04'877"E
6	Çanakkale (Central)	Dardanos Municipality Public Beach	40°05'250"N - 26°21'735"E
7	Çanakkale (Central)	Güzelyalı Municipality Public Beach	40°02'950"N - 26°20'578"E
8	Çanakkale (Central)	Yeni Kordon Bathing Water Site	40°08'285"N - 26°23'890"E
9	Eceabat	Eceabat Bathing Water Site	40°10'690"N - 26°21'800"E
10	Eceabat	Kabatepe Recreational Coastal Water	40°11'825"N - 26°15'980"E
11	Ezine	Geyikli Odunluk Pier Recreational Coastal Water	39°46'720"N - 26°09'385"E
12	Gelibolu	Güneyli Bathing Water Site	40°30'610"N - 26°41'640"E
13	Gökçeada	Aydincik Recreational Coastal Water	40°07'470"N - 25°56'070"E
14	Gökçeada	Yıldız Bay Recreational Coastal Water	40°14'110"N - 25°54'230"E

Bacterial isolation and identification

One milliliter of each seawater sample was serially diluted and spread on Marine Agar (Difco™) using the surface plating method. Plates were incubated at $22 \pm 0.1^\circ\text{C}$ for 72 hours, and colony forming was monitored daily.

Based on colony morphology, representative isolates were selected and subjected to Gram staining. Isolates were then identified to the species level using the VITEK® 2 Compact 30 microbial identification system (bioMérieux, France). Pure cultures of all isolates were maintained for subsequent antibiotic susceptibility testing.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method. The following antibiotic disks were used in this study. The antibiotic derivatives and doses are summarized in Table 2.

Bacterial suspensions were adjusted to 0.5 McFarland standard and spread evenly on Mueller-Hinton Agar

(Oxoid™). Antibiotic disks were placed at equal distances, and plates were incubated at $37 \pm 0.1^\circ\text{C}$ for 24 hours.

The inhibition zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines. Each isolate was classified as Resistant (R), Intermediate (I), or Susceptible (S). *Escherichia coli* ATCC 25922 was used as a reference strain for quality control.

Calculation of MAR (multiple antibiotic resistance) index

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate using the formula defined by Krumpman (1983): $\text{MAR index} = \text{Number of antibiotics to which the isolate is resistant} / \text{Total number of antibiotics tested}$

Isolates with a MAR index ≥ 0.2 were considered to originate from high-risk environments with frequent exposure to antibiotics.

Table 2. Antibiotic derivatives and doses used in the tests.

Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)
Amoxycillin	AMC	30
Ampicillin	AMP	10
Ceftazidime	CAZ	30
Kanamycin	K	5
Vancomycin	VA	30
Aztreonam	ATM	30
Cefotaxime	CTX	30
Cefuroxime	CXM	30
Gentamycin	CN	120
Imipenem	IPM	10
Ofloxacin	OFX	5
Oxytetracycline	OT	30
Rifampicin	RA	2
Sulphonamides compound	SE	300
Tetracycline	TE	30

Results and Discussion

Antibiotic resistance

A total of 211 bacterial isolates were obtained from the coastal waters of the Dardanelles Strait and evaluated for their resistance profiles against 15 distinct antibiotics. The findings indicated notably high resistance levels, particularly to vancomycin (92.89%), kanamycin (81.04%), sulphonamides (64.45%), and ampicillin (51.18%). In contrast, ceftazidime exhibited the lowest resistance rate (13.27%), suggesting it remains relatively effective in marine bacterial populations. These observations highlight a concerning decline in the effectiveness of widely used antibiotics in coastal aquatic environments.

Resistance frequencies of the isolates against the 15 antibiotics were analyzed (Table 2). The highest percentage values among the categories (R: resistant, S: susceptible, I: intermediate) for each antibiotic are presented in bold. Vancomycin resistance was the most prevalent, reaching 92.89%, while ceftazidime showed the least resistance across all isolates. The highest intermediate response was 36% for rifampicin, whereas the lowest was 4% for ceftazidime. The greatest rate of susceptibility was found to be 81.99% for ceftazidime, while vancomycin, kanamycin, and sulphonamides had the lowest susceptibility levels, each at just 1%.

Several of the bacterial species isolated in this study are well-known environmental or opportunistic pathogens

and have been frequently reported in marine ecosystems. For example, *Acinetobacter* spp. (especially *A. baumannii*, *A. haemolyticus*, and *A. nosocomialis*) are often found in marine waters and even fish viscera. These species are of global concern due to their multidrug resistance, including resistance to carbapenems, a last-resort antibiotic group (Dewi et al., 2021).

A. baumannii is frequently isolated from various marine environments, including seawater, marine sediments, and fish viscera. Studies show that environmental strains of this species can survive in seawater for up to 10 days and are more prevalent in coastal areas exposed to organic pollution (Twala, 2023). Although primarily known as a hospital-associated species, *A. nosocomialis* has also been identified in marine and estuarine environments. Coastal studies from South Korea have isolated this species from seawater, especially in transitional zones between freshwater and saltwater (Adewoyin et al., 2018). These findings suggest that *A. nosocomialis* can adapt to dynamic aquatic ecosystems and may serve as a reservoir for resistance genes (Sanz-García et al., 2021). While less frequently studied, *A. haemolyticus* has occasionally been recovered from coastal waters, particularly in areas impacted by urban wastewater. Its detection in marine samples near outfall zones suggests a possible link to human-derived contamination and points to its ability to persist in saline aquatic environments (Van Assche, 2019).

Table 3. The isolate codes and tested bacteria species

Bacteria species	Isolation Frequency (%)	Station Code
<i>Acinetobacter baumannii</i> complex (Bouvet and Grimont 1986)	1.14	St 4
<i>Acinetobacter haemolyticus</i>	2.27	St 5, St 6
<i>Acinetobacter nosocomialis</i>	2.27	St 1, St 6
<i>Bacillus mycoides</i> Flüge 1886	1.14	St 6, St 8, St 10
<i>Bacillus pumilus</i> Meyer and Gottheil 1901	2.27	St 6, St 7, St 8
<i>Bacillus thuringiensis</i> Berliner 1915	2.27	St 6, St 7, St 8
<i>Bacillus cereus</i> Frankland and Frankland 1887	5.68	St 3, St 6, St 7, St 8
<i>Bacillus megaterium</i> de Bary 1884	2.27	St 6, St 7, St 8
<i>Burkholderia mallei</i> (Zopf 1885) Yabuuchi et al. 1993	9.09	St 6, St 9, St 10, St 14
<i>Citrobacter braakii</i> Brenner et al. 1993	6.82	St 6, St 7, St 8
<i>Citrobacter freundii</i> (Braak 1928) Werkman and Gillen 1932	5.68	St 10, St 13, St 14,
<i>Enterobacter aerogenes</i> Hormaeche and Edwards 1960	7.95	St 11, St 7, St 8
<i>Enterobacter cloacae</i> (Jordan 1890) Hormaeche and Edwards 1960	10.23	St 13, St 7, St 8
<i>Enterobacter sakazakii</i> (Farmer et al. 1980)	2.27	St 4, St 7, St 8
<i>Enterococcus faecalis</i> (Andrewes and Horder. 1906) Schleifer and Kilpper-Bälz. 1984	10.23	St 10, St 12, St 14,
<i>Escherichia coli</i> (Migula 1895) Castellani and Chalmers 1919	28.41	St 5, St 7, St 8
<i>Klebsiella ornitholytica</i>	6.82	St 4, St 7, St 8
<i>Klebsiella oxytoca</i> (Flüge 1886) Lautrop 1956	4.55	St 1, St 5
<i>Klebsiella pneumoniae</i> (Schroeter 1886) Trevisan 1887	5.68	St 6, St 7, St 8
<i>Kocuria kristinae</i>	1.14	St 2
<i>Pantoea agglomerans</i> (Ewing and Fife 1972) Gavini et al. 1989	5.68	St 2, St 9, St 13, St 13
<i>Proteus mirabilis</i> Hauser. 1885	4.55	St 6, St 7, St 8, St 11
<i>Proteus vulgaris</i> Hauser. 1885	3.41	St 4, St 13
<i>Pseudomonas fluorescens</i> Migula 1895	3.41	St 6, St 7, St 8
<i>Pseudomonas stutzeri</i>	1.14	St 13
<i>Raoultella planticola</i> (Bagley et al. 1982) Drancourt et al. 2001	2.27	St 4, St 12
<i>Staphylococcus intermedius</i> Hajek 1976	11.36	St 6, St 7, St 8
<i>Salmonella enterica</i> Le Minor & Popoff 1987	3.41	St 6, St 7, St 8
<i>Serratia marcescens</i> Bizio 1823	4.55	St 14, St 5
<i>Serratia plymuthica</i> (Lehmann and Neumann 1896) Breed et al. 1948	5.68	St 9, St 13, St
<i>Sphingomonas paucimobilis</i> (Holmes et al. 1977) Yabuuchi et al. 1990	18.18	St 3, St 6, St 7, St 8
<i>Staphylococcus pseudintermedius</i> Devriese et al. 2005	2.27	St 11, St 2
<i>Stenotrophomonas maltophilia</i> Hugh 1981) Palleroni and Bradbury 1993	12.50	St 6, St 7, St 8, St 9, St 11
<i>Streptococcus uberis</i> Diernhofer 1932 (Approved Lists 1980)	1.14	St 13
<i>Vibrio alginolyticus</i> (Miyamoto et al. 1961) Sakazaki 1968	1.14	St 10

Bacillus species such as *B. cereus*, *B. pumilus*, *B. thuringiensis*, and *B. mycoides* are prevalent in marine sediments and contribute to organic matter turnover through extracellular enzyme activity. However, *B. cereus*, in particular, is known for its toxigenic potential and resistance traits, making it relevant for both food safety and environmental monitoring (Das et al., 2006).

Pseudomonas spp. including *P. fluorescens* and *P. stutzeri* are metabolically versatile and widely distributed in seawater and coastal sediments. These bacteria play key roles in nitrogen cycling and hydrocarbon degradation and often harbor plasmid-mediated resistance genes (Das et al., 2024).

Vibrio alginolyticus, a species common in warm, saline waters, acts both as a free-living marine bacterium and as a fish pathogen. It has been found to exhibit resistance to antibiotics like tetracycline and ampicillin in estuarine and polluted coastal areas (Toraskar, et al., 2022).

Members of the Enterobacteriaceae family such as *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Escherichia* are commonly detected in coastal waters influenced by wastewater discharge. These genera often indicate fecal

contamination and show high rates of extended-spectrum beta-lactamase (ESBL) production and multidrug resistance (Dewi et al., 2020).

Other less frequently reported but environmentally significant bacteria like *Stenotrophomonas maltophilia*, *Sphingomonas paucimobilis*, and *Raoultella planticola* were also detected in this study. These organisms, although isolated in low abundance, are known to carry resistance determinants and adapt to polluted marine niches. Similarly, *Enterococcus faecalis* and *Staphylococcus pseudointermedius* are indicators of human or animal waste contamination and may persist in shoreline environments.

Overall, the bacterial community identified from the coastal waters of the Çanakkale Strait includes numerous species previously associated with antibiotic resistance in marine settings. This highlights the importance of continuous environmental surveillance and reinforces the role of coastal waters as reservoirs of resistance. The widespread occurrence of isolates with MAR index ≥ 0.2 strongly supports the hypothesis that these environments are heavily impacted by anthropogenic antibiotic inputs and serve as active reservoirs for resistant bacteria.

Table 4. The antibiotic resistance frequencies of the bacterial isolates

	Number of isolates			Frequency (%)		
	R	S	I	R	S	I
Amoxycillin (AMC 30 µg)	75	80	56	35.55	37.91	26.54
Ampicillin (AMP 10 µg)	108	57	46	51.18	27.01	21.80
Ceftazidime (CAZ 30 µg)	28	173	10	13.27	81.99	4.74
Kanamycin (K 5 µg)	171	16	24	81.04	7.58	11.37
Vancomycin (VA 30 µg)	196	3	12	92.89	1.42	5.69
Aztreonam (ATM 30 µg)	63	127	21	29.86	60.19	9.95
Cefotaxime (CTX 30 µg)	35	152	24	16.59	72.04	11.37
Cefuroxime (CXM 30 µg)	97	67	47	45.97	31.75	22.27
Gentamycin (CN 120 µg)	44	107	60	20.85	50.71	28.44
Imipenem (IPM10 µg)	32	148	31	15.17	70.14	14.69
Ofloxacin (OFX 5 µg)	47	145	19	22.27	68.72	9.00
Oxytetracycline (OT 30 µg)	34	108	69	16.11	51.18	32.70
Rifampicin (RA 2 µg)	92	41	78	43.60	19.43	36.97
Sulphonamides compound (S3 300 µg)	136	36	39	64.45	17.06	18.48
Tetracycline (TE 30 µg)	100	91	20	47.39	43.13	9.48

R: Resistance S: Susceptible I: Intermediate

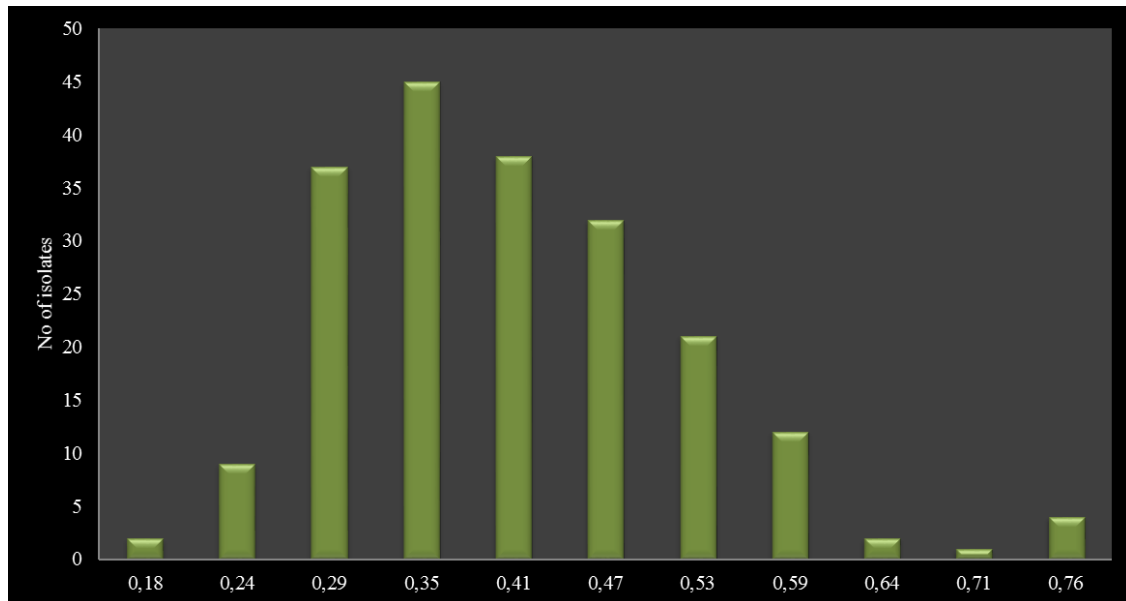


Figure 2. Distribution of multiresistant bacterial isolates according to stations

Multiple antibiotic resistance

Figure 2 summarizes bacterial isolates multiple antibiotic resistance (MAR). The multiple antibiotic resistance (MAR) index values of the bacterial isolates were recorded as 0.35 (95%), 0.29 (88%), 0.64 (853) and 0.71 (47%).

Investigating bacterial diversity within marine ecosystems is essential for understanding environmental processes and determining sources of pollution. Due to the fluctuating nature of marine environments—unlike the relative stability of terrestrial habitats—bacterial communities evolve resistance strategies to cope with ongoing environmental pressures. The detection of resistant strains in these systems points to continuous exposure to antibiotic and chemical pollutants (Zeglin, 2015; Delgado-Baquerizo, 2016).

The inappropriate use of antibiotics continues to be a major risk factor for both human health and ecological integrity. Aquatic systems, which are significantly influenced by human activity, act as hotspots for the development and spread of antimicrobial resistance. While there is growing interest in the discovery of novel antibiotic compounds, pharmaceutical research and development in this field is often limited by economic constraints. As such, the implementation of strong policies to curb the unnecessary use of antibiotics has become increasingly critical. Antibiotics reach marine systems through domestic, industrial, and healthcare-related discharges, and facilitate the horizontal transmission of resistance genes among microbial populations. Since bacteria readily adhere to surfaces, coastal zones offer ideal conditions for exchanging resistance determinants developed in response to environmental stressors like antibiotics and heavy metals (Sabatino et al., 2020; Zhang et al., 2020; Marti et al., 2014).

Numerous studies have confirmed antibiotic and heavy metal resistance among bacterial isolates from Turkish marine sediments. For instance, Matyar et al. (2008) documented that isolates from Iskenderun Bay sediment displayed high resistance to ampicillin (94.4%) and lower resistance to imipenem (4.4%). In Güllük Bay, Altuğ et al. (2020) observed that sediment-derived bacteria were entirely resistant (100%) to sulfonamide, rifampicin, tetracycline, and ampicillin, and showed near-complete resistance to nitrofurantoin (98%) and oxytetracycline (98%). Similarly, Kacar and Kocyigit (2013) reported gentamicin and tobramycin resistance among bacteria from sediments collected at the Aliğa shipbreaking zone in the Eastern Aegean. Çardak et al. (2016) identified high resistance levels in isolates from the Marmara Sea and Turkish Straits—specifically to kanamycin (82%), vancomycin (78%), and ampicillin (60%).

The findings of the current research also demonstrate considerable resistance in marine bacteria isolated from Çanakkale's coastal waters, especially against vancomycin (93.3%), kanamycin (81.04%), sulphonamides (64.45%), ampicillin (51.1%), tetracycline (47.39%), cefuroxime (45.97%), and ceftazidime (13.27%). These outcomes suggest that even locations perceived as relatively clean, such as the Çanakkale Strait, may harbor higher-than-expected levels of antibiotic contamination. Consequently, environmental monitoring, particularly in areas with heavy maritime traffic and recreational use, is strongly advised.

The MAR (Multiple Antibiotic Resistance) index values ranged from 0.14 to 0.71. Notably, over 95% of the isolates had a MAR index ≥ 0.2 . According to Krumpertman's criteria, MAR values above 0.2 are indicative of high-risk contamination sources, likely from anthropogenic activities. The widespread nature of these elevated MAR values along the Çanakkale coast

underscores the persistent impact of untreated or partially treated urban wastewater, maritime discharges, and seasonal tourism activities.

Average antibiotic resistance across all isolates was calculated at 95.46%. This is consistent with findings in urban-impacted regions. Heterotrophic aerobic bacteria exhibited 100% resistance, followed by enterococci and coliform groups. These bacteria, typically indicators of fecal pollution, are known to acquire resistance genes rapidly in polluted environments.

Comparable studies further validate these observations. For instance, in Gökçeada, sulphonamide resistance reached 93.3%, and cefotaxime resistance 78.9%, mirroring the high resistance seen in the current data. Research in Istanbul's Golden Horn reported ampicillin (71.5%), sulphonamides (43.8%), and rifampin (24.3%) resistance among 144 isolates, again indicating strong antibiotic pressure in urban estuaries. Interestingly, gentamicin and imipenem had 0% resistance there, a trend similar to the low resistance.

In another investigation conducted in Kınalı Island, nearly all isolates (95.46%) were resistant to antibiotics, with oxytetracycline and tetracycline showing the highest resistance (98.7%). Even nitrofurantoin, typically considered a last-resort antibiotic, showed 87.3% resistance (Türetken et al., 2024), raising concerns about limited therapeutic options.

Aegean Sea sediment isolates exhibited relatively lower resistance to amikacin (36%), cefotaxime (20%), ampicillin (16%), and kanamycin (16%). The elevated resistance rates in Çanakkale likely reflect the difference between enclosed coastal waters affected by urban runoff and the more open, less polluted marine sediments.

Of particular note is the frequent identification of *Bacillus cereus* in the current samples, a bacterium recognized in both Aegean and Gökçeada datasets as one of the most multidrug-resistant. Its recurrence across studies confirms the need for targeted monitoring of key resistant species in marine surveillance programs.

The role of marine environments as reservoirs for resistance genes has been repeatedly emphasized. While aquaculture sites are often scrutinized for their role in antibiotic release, recreational zones like Çanakkale pose direct threats to public health. The detection of multidrug-resistant bacteria in waters used for swimming and fishing increases the likelihood of exposure for humans, especially immunocompromised individuals (Al-Bahry et al., 2009; Di Cesare et al., 2012; Altuğ et al., 2020).

Furthermore, the high MAR values in this study surpass those reported in other Turkish regions. For example, bacteria isolated from the Sea of Marmara had MAR indices ranging from 0.30 to 0.34 (Çardak et al., 2016), whereas in this study, MAR values reached up to 0.71. This points to a greater cumulative impact of urbanization and shipping traffic in Çanakkale,

emphasizing the urgent need for stricter discharge regulations and continuous microbial water quality assessments.

In terms of aminoglycoside resistance patterns, Çanakkale isolates exhibited higher resistance to kanamycin compared to amikacin. This suggests possible selective antibiotic exposure, where local medical waste or domestic discharges may contain specific compounds that promote kanamycin resistance. In contrast, amikacin resistance dominates in sediment samples from aquaculture zones, hinting at differences in antibiotic usage practices.

Overall, the findings of this study highlight a serious concern: coastal ecosystems, particularly those near urban centers, are increasingly burdened with antibiotic-resistant bacteria. The elevated resistance frequencies, high MAR indices, and cross-regional comparisons all reinforce the necessity for integrated marine management policies, enhanced wastewater treatment infrastructure, and routine antimicrobial surveillance programs.

In this study, it was observed that bacterial resistance to antibiotics in the coastal waters of the Çanakkale Strait likely developed through spontaneous mutations or via the horizontal transfer of resistance genes from other bacterial populations. Exposure to antibiotic residues in the aquatic environment has created a selective pressure that favors the survival and proliferation of resistant strains, allowing them to dominate local microbial communities. This pattern reinforces the understanding that environmental antibiotic pollution plays a central role in the emergence and spread of antimicrobial resistance within aquatic ecosystems.

Although the “One Health” approach has increased interdisciplinary awareness regarding the connections between environmental, human, and animal health, antibiotic resistance remains a serious and growing concern. Marine inventory studies, particularly in urban and tourism-affected regions like Çanakkale, are vital to track the spread of resistance genes and better understand the dynamics of contamination.

The distribution and density of antibiotic-resistant bacteria vary depending on geographic exposure levels, yet aquatic environments consistently contribute to the persistence and transmission of resistance. The findings from Çanakkale support the need for ongoing monitoring programs in coastal zones that face multiple anthropogenic pressures.

The elevated levels of resistance detected for the first time in this region, particularly along the Çanakkale coastline, underscore the necessity of regular and long-term microbial assessments. These results not only reflect current contamination patterns but also highlight potential risks to both marine ecosystems and public health, especially in the context of climate change and seasonal urban activity.

Conclusion

The present study provides the first detailed account of antibiotic resistance in bacterial populations isolated from the coastal waters of the Çanakkale Strait. The high frequency of resistance, coupled with widespread multidrug resistance patterns and elevated MAR index values, clearly demonstrates the anthropogenic pressures affecting the region. These findings confirm that the Çanakkale coastline has become a local reservoir for antibiotic-resistant bacteria, likely driven by urban discharge.

This study contributes valuable baseline data to the national marine antibiotic resistance inventory. However, to fully understand the evolving threat and its ecological implications, continuous and expanded monitoring efforts are required. Future research should integrate environmental variables such as seasonal changes and pollution sources, and adopt multidisciplinary frameworks that can inform policy and guide sustainable coastal management practices in Türkiye and beyond.

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Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article. No funding was received for conducting this study. The authors declare they have no financial interests.

Author Contributions

The conception and design of the study were carried out by the first author, Belgin Kılıç Çetinkaya. The manuscript was written by Belgin Kılıç Çetinkaya and revised with contributions and feedback from the second author, Mine Çardak. Both authors read and approved the final version of the manuscript.

Ethics Approval

The authors declare that this study did not include any experiments with human or animal subjects.

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