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Research Article

Frequency of Antibiotic-Resistant Bacteria isolated from the Kınalıada Coastal Areas of the Sea of Marmara, Türkiye

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ABSTRACT

In the process of global climate change, the negative effects of anthropogenic activities on microbial interactions have become more visible in coastal areas. Because island coastal ecosystems are fragile ecosystems that are open to dynamic environmental variables, it is important to determine bacteriological signals in these regions. The frequency of bacterial antibiotic resistance in aquatic ecosystems is a micro-marker of human activity. The frequency of antibiotic-resistant bacteria was investigated in surface water samples collected from the coastal areas of Kınalıada Island in the Sea of Marmara between 2018 and 2019. The bacteria isolated from the sea water were screened against: spectinomycin (SC300), nitrofurantoin (F50), Rifampicin (Rd2), tetracycline (TE30), ampicillin (AMP10), and oxytetracycline (OT30) using the disk diffusion technique. The frequencies of antibiotic-resistant faecal coliform, total coliform, intestinal enterococcus, and heterotrophic aerobic bacteria were evaluated according to the Clinical Laboratory Standard Institute (CLSI). The antibiotics to which all bacterial isolates showed the highest resistance were tetracycline and oxytetracycline (98.7% The frequency of resistant heterotrophic aerobic bacteria was recorded at 100% against all tested antibiotics. All bacterial isolates showed resistance to more than three antibiotic derivatives, and the Multiple Antibiotic Resistance (MAR) index was determined to be in the range of 0.67–1. The findings of this study provide regional evidence of the influence of anthropogenic pollution on the spread of antibiotic resistance. The detection of high levels of antibiotic-resistant bacteria indicated that the coastal areas of Kınalıada are at potential risk for the global spread of resistant bacteria, human health, and ecosystem function.

Keywords: The Sea of Marmara, Kınalıada Island, bio-indicator bacteria, antibiotic resistant bacteria

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INTRODUCTION

The marine environment harbours bacteria that play an important role in the decomposition of organic matter in the ecosystem, as well as pathogenic bacteria that enter the marine environment via human activities. Antibiotic pollution caused by anthropogenic activities domestic and industrial wastewaters, and livestock farming, cause the spread of bacterial antibiotic resistance in aquatic areas. Among these pollutants, antibiotic derivatives regional antibiotic resistance in aquatic areas serves as a reservoir for the spread of global bacterial resistance.

The occurrence of antibiotic-resistant bacteria in aquatic areas has been documented to be associated with anthropogenic activities (Yang et al., 2013; Terzi and Isler 2019). Furthermore, altered bacterial metabolism under the influence of environmental stress favours the spread of antibiotic-resistant pathogens due to global climate change (MacFadden et al., 2018, Cavicchioli et al., 2019).

Consideration of the relationship between humans, animals, and the environment to prevent the global problem of antimicrobial resistance (AMR) is increasingly recognised worldwide.

Guidelines explaining this in a one-health approach have been strengthened by scientific studies on this topic (McEwen and Collignon 2018; Rüegg et al., 2018; Larsson et al., 2023). Bacterial resistivity data are important for defining the global health dimension of the regular functioning of marine ecosystems. Marine environments offer a unique laboratory for understanding how microorganisms will be affected by global climate change and increasing human activities (Cavicchioli et al., 2019). The natural environment is a genetic resource for the global distribution of antibiotic-resistant bacteria, which poses a threat to humans, animals, and certain crops in the treatment regime of infectious diseases and thus hosts the transition of susceptible microorganisms to resistance (Larsson et al., 2023). The presence of bacteria in the marine environment is not stable and varies according to the conditions. Bacteria can grow in different environments, such as seawater, sediment, and the biota, according to their life requirements. Both planktonic and attached bacteria may change their number in seawater due to the effects of human activities. In marine environments, bacteria can develop different resistance mechanisms and adaptations according to the pollutants they are exposed to. Heterotrophic bacteria convert organic matter into forms that other organisms can use to generate energy and metabolic processes. Intensive human activities in coastal areas create a favourable environment for bacterial growth. The presence of indicator bacteria in marine coastal areas varies depending on the success of anthropogenic pollution control.

However, higher numbers of indicator bacteria were reported in coastal areas. (Fernandes Cardoso de Oliveira 2010; Cicin-Sain et al.2011).

The spread of antibiotic-resistant bacteria in natural environments leads to the ineffective use of antibiotics for the treatment of infectious diseases. The increase in global antibiotic resistance triggers a vicious cycle of continuous development of new antibiotic derivatives. Therefore, it is important to determine the frequency of bacterial antibiotic resistance characterised by coastal areas, especially considering that aquatic areas are reservoirs for the global distribution of antibiotic resistance. The Sea of Marmara is under the effects of various environmental pressures and is one of the most important waterways in the world. Ship ballast water is one such pressure, and high antibiotic resistance has been reported in bacteria isolated from the ballast tanks of ships entering the Marmara Sea from different geographical areas (Altuğ et al., 2012). The frequency of antibiotic-resistant bacteria in the Sea of Marmara, particularly in the coastal area of Istanbul Province, has been reported in different studies. The data obtained indicate the presence of antibiotic-resistant bacteria in the Turkish Strait System, including the Istanbul Strait (Bosphorus) and Çanakkale Strait (Dardanelles), which connect the Marmara Sea to the Black Sea and Mediterranean (Altuğ and Balkıs 2009; Sivri and Akbulut 2016; Çardak et al., 2016; Kimiran et al 2007).

Island ecosystems have a more sensitive position as specialised ecosystems in marine environments. In a previous study conducted on seawater samples taken from the coastal areas of Kınalıada, the first bacteriological data on indicator bacteria, which provide clues to the presence of pathogenic bacteria, were reported above national and international standards (Karaman-Baş and Altuğ, 2022). Several daily island ferry movements from Istanbul increase the population density of the region during the summer months. This situation adds to the fragile structure of the region.

In this study, the frequency of antibiotic-resistant faecal coliforms, total coliforms, intestinal enterococci, and heterotrophic aerobic bacteria against spectinomycin, nitrofurantoin, rifampicin, tetracycline, ampicillin, and oxytetracycline was investigated in surface water samples taken from Kınalıada coastal areas to investigate land-based pollution effects on the island ecosystem in the period between June 2018 and May 2019.

MATERIAL AND METHODS

Study area

The group of islands in the province of Istanbul consists of nine separate islands called the Prince Islands. Büyükada, Heybeliada, Burgazada, and Kınalıada are inhabited, and these islands host intensive touristic activities in the summer months. Kınalıada Island, among the Prince Islands hosted by the Sea of Marmara, is the closest island to Istanbul. The coastal area of Kınalıada, which is located in the Sea of Marmara, hosts domestic and industrial activities as well as intensive touristic use for swimming and boat tourism in spring and summer. Kınalıada is located 7 miles south of Istanbul.

Sampling

Seawater samples were collected under aseptic conditions in brown sterile glass bottles that do not transmit sunlight and were delivered to the laboratory on the same day using a cold chain (APHA, 2012). Surface water (0-30 cm) sampling was carried out monthly in summer months (June 2018, July 2018, August 2018) when bacterial activity was high, and seasonally in autumn (November 2018), winter (February 2019), and spring (May 2019) at ten stations of Kınalıada Island, the Sea of Marmara. Sampling was carried out within the scope of the project titled "Investigation of Bio-Indicator Bacteria Levels in Kınalıada Coastal Area" (Karaman-Baş and Altuğ 2022).

The sampling stations in the study areas are shown in Figure 1.

The locations of the stations are listed in Table 1.

Isolation of heterotrophic aerobic bacteria (HAB) and bio-indicator bacteria

Bacterial antibiotic resistance was tested in heterotrophic aerobic bacteria and indicator bacteria (faecal coliform, total coliform and faecal and intestinal enterocci) isolated from the surface water of the coastal area of Kınalıada Island. Heterotrophic aerobic bacteria isolation were performed according to the method of Austin (1988); bioindicator bacteria analyses consisting of faecal coliform, total coliform, and intestinal enterococcus were performed according to APHA (2012), as detailed in our previous study (Karaman Baş, Altuğ 2022).

Frequency of antibiotic-resistant bacteria

In the antibiogram test Oxoid (UK) discs of spectinomycin (SC300), nitrofurantoin (F50), rifampicin (RD2), tetracycline (TE30), ampicillin (AMP10), and oxytetracycline (OT30) were used. The antibiotic derivatives and doses is summarised in Table 2.

The frequency of antibiotic-resistant bacteria was investigated using the Kirby-Bauer disk diffusion method. The selected 2-3 colonies from dishes were maintained at room temperature for 5–10 min and spread on the surface of Petri dishes containing Mueller-Hinton Agar. Discs containing different antibiotics at various concentrations were placed on the surface of the agar with sterile forceps and incubated at 25 °C for heterotrophic aerobic bacteria and at 37 °C for 24-48 hours for faecal coliform, total coliform, and intestinal enterococci. At the end of incubation, the diameters of the zones around the disks were measured millimetrically to determine whether they were resistant, sensitive, or suspicious. Isolates with full growth on the medium were recorded as resistant. Isolates that formed a clear zone around the disk were recorded as antibiotic-sensitive or susceptible. The plate observations were evaluated in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2017).

The percentage distribution of the number of resistant isolates according to the total number of isolates was calculated, and the results are presented as "% frequency of antibiotic-resistant bacteria". In total, 150 bacterial isolates, including total coliforms, faecal coliforms, and intestinal enterocoliforms enterococci, were tested.

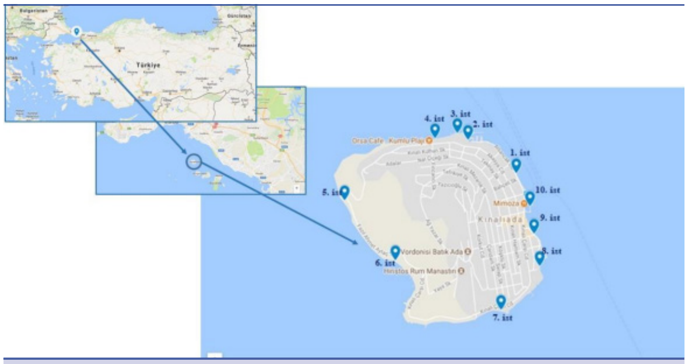

Figure 1. Study Area (Google Maps).

Multiple Antibiotic Resistance (MAR)

The resistance of the isolates to more than one antibiotic was calculated using the multiple antibiotic resistance (MAR) index using the following equation.

MAR index = a/bxc

a = total antibiotic resistance score of all isolates;

b = total number of isolates

c = number of isolates in the sample (Krumperman, 1983).

Bacterial isolates resistant to three or more antibiotic derivatives were defined as multi- antibiotic resistant (ranging from two to ten).

RESULTS AND DISCUSSION

The distribution of antibiotic resistance of bacteria isolated from seawater by faecal coliform, total coliform, intestinal enterococci, and heterotrophic aerobic bacteria, percent resistance of bacteria by antibiotic derivative, and MAR index data showing multiple antibiotic resistance are presented below. The numbers of faecal coliform, total coliform, intestinal enterococci, and heterotrophic aerobic bacteria in the strains tested against the six antibiotic derivatives are shown in Table 3.

The mean antibiotic resistance frequency of all isolates was 95.46%. Heterotrophic aerobic bacteria showed the highest antibiotic resistance (100%) followed by intestinal enterococci, total coliform, and faecal coliform bacteria.

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

Station No	Sampling Name	Latitude-Longitude	
1	Public Beach I	*40°91'27.39"N*	*29°05'28.87"E*
2	Water Club Port Inner	*40°91'40.42"N*	*29°05'05.23"E*
3	Water Club Pier	*40°91'45.53"N*	*29°05'07.27"E*
4	Public Beach II,	*40°91'40.99"N*	*29°04'80.34"E*
5	Reference Station	*40°91'02.39"N*	*29°04'01.16"E*
6	Special Beach I:	*40°90'64.43"N*	*29°04'43.97"E*
7	26 Number Beach,	*40°90'34.52"N*	*29°05'32.91"E*
8	Special Beach II	*40°90'62.18"N*	*29°05'65.74"E*
9	Kınalıada-Police Centre	*40°90'80.91"N*	*29°05'61.45"E*
10	Marine Taxi Pier	*40°90'99.02"N*	*29°05'57.59"E*

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

Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)	Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)
Ampicillin	AMP10	10	Nitrofurantoin	F50	50
Spectinomycin	SC300	300	Rifampicin	RD2	2
Tetracycline	TE30	30	Oxytetracycline	OT30	30

Resistance reactions of bacteria to the six tested antibiotic derivatives are shown in the Table 4.

Bacterial antibiotic resistance varied from 87.3% to 98.7%. The highest resistance was recorded against tetracycline and oxytetracycline (98.7%. The highest frequency of antibiotic-resistant faecal coliform was recorded against ampicillin and tetracycline at 100%, whereas the highest frequency of antibiotic-resistant total coliform was recorded against oxytetracycline at 100%. The highest frequency of antibiotic-resistant intestinal enterococci was recorded for oxytetracycline and spectinomycin. The frequency of antibiotic-resistant heterotrophic aerobic bacteria was 100% against all tested antibiotics.

The frequency of bacteria resistant to specific antibiotics (%) in seawater is shown in Figure 2.

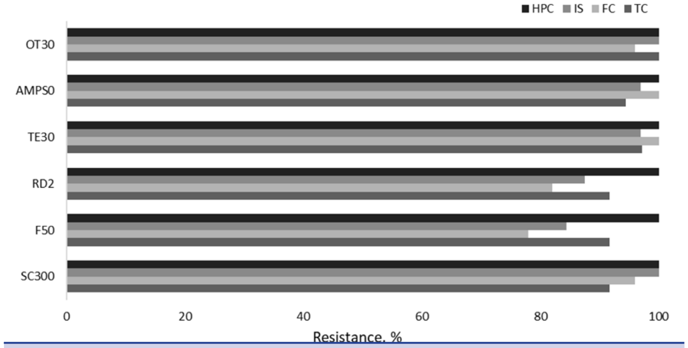

Figure 2. The frequency of bacteria resistant to certain antibiotics (%) in seawater.

95.46% of the 150 isolates were resistant to the tested antibiotics. Isolates showed resistance between 98% and 87.3%. The antibiotics to which the isolates showed the highest resistance (98.7%) were oxytetracycline and tetracycline. The lowest resistance was observed against nitrofurantoin (87.3%. Multiple antibiotic resistance indexes and resistance ratios are presented in Table 5.

The multiple antibiotic resistance (MAR index) of each antibiotic-resistant bacteria was calculated. A MAR index lower than 0.2 indicates a nonpoint source of pollution. A MAR index >0.2 indicates a specific pollution focus and high risk of antibiotic contamination (Nies,1999). In this study, all bacterial isolates exhibited resistance to more than three antibiotic derivatives, and the MAR index was determined to be in the range of minimum 0.67 to maximum 1.

Studies conducted in marine environments across different geographical regions revealed the existence of a marine antibiotic resistance reservoir. While studies conducted around fish farms pose a potential risk for aquatic food supply security, in areas used for recreational purposes, this situation has emerged as a threat affecting human health and, basically, ecosystem functions (Al-Bahry et al., 2009; Fernandes Cardoso de Oliveira et al., 2010; Di Cesare et al. 2012; Mulamattathil et. al., 2014; Altuğ et al., 2020). Antibiotic resistance is therefore one of the greatest challenges facing modern medicine worldwide (Andersson et al., 2020). In this study, the recorded frequency of resistance of bacteria isolated from seawater samples against specific antibiotics showed a high distribution in the range of 5%–50%. This high frequency of resistance detected at the local level indicates once again the importance of protecting the seas, which are reservoirs in the global distribution of antibiotic resistance, from the pressure of human activities.

Table 3. Numbers of antibiotic-resistant faecal coliform (FC), total coliform (TC), and intestinal enterococcus (IE), heterotrophic aerobic bacteria (HAB), and mean resistivity frequency (%).

Bacteria	Number of strains tested	Mean resistivity frequency %
FC	50	92.00
TC	36	94.40
IE	32	94.78
HAB	32	100
Total number of strains	150	95.46

Table 4. Resistance reactions of bacteria to the tested antibiotic derivatives.

*Antibiotics	Dose (µg)	TC (N=36)	FC (N=50)	IE (N=32)	HAB (N=32)	Resistant Bacteria (Σ)	Resistance (%)
		Numbers of Resistant Bacteria					
OT30	30	36	48	32	32	148	98.7
AMP10	10	34	50	31	32	147	98.0
TE30	30	35	50	31	32	148	98.7
RD2	2	33	41	28	32	134	89.3
F50	50	33	39	27	32	131	87.3
SC300	300	33	48	32	32	145	96.7

*OT30: oxytetracycline, AMP10: ampicillin, TE30: tetracycline, RD2: rifampicin, F50: nitrofurantoin, SC300: spectinomycin. TC: Total Coliforms, FC: Faecal Coliforms IE: Intestinal Enterococci, HPC: Heterotrophic Aerobic Bacteria

Table 5. Multiple Antibiotic Resistance Indexes and Resistance Ratios of Isolates.

Number of antibiotics	Numbers of bacteria resistant to antibiotics	MAR Index	Resistance %	p-value
1	0	0.00	0.00	0.00
2	0	0.00	0.00	0.00
3	0	0.00	0.00	0.00
4	13	0.67	8.67	0.1625
5	21	0.83	14.00	0.3935
6	116	1.00	77.33	0.4384

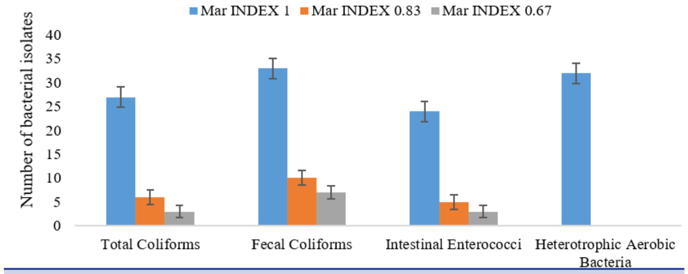

Figure 3. Multiple Antibiotic Resistance Levels of TC, FC, IE and HAB.

In several studies antibiotic resistant bacteria data were reported from various parts of the Sea of Marmara (Altuğ and Balkıs 2009; Sivri and Akbulut 2016; Çardak et al., 2016; Kimiran et al 2007). The MAR index of bacteria isolated from the Sea of Marmara was reported to be between 0.30 and 0.34, which was higher than that of the isolates obtained from the Canakkale Strait (Çardak et al., 2016). In this study, the first regional MAR value was detected to be at least two times higher than that in previous studies. This situation constitutes evidence of the anthropogenic pressure on the Kınalada coastal area of Kınalıada is exposed in terms of bacterial antibiotic resistance.

CONCLUSION

A spontaneous or induced mutations or by the transfer of resistance genes from other bacteria, acquired genes responsible for the development of resistance to antibiotics in bacteria. In the case of exposure to antibiotics, resistance genes are naturally selected because bacteria carrying them have a better chance of survival, and the space occupied by bacteria carrying them in the ecosystem increases. Aquatic areas are considered reserves in the distribution of global antibiotic resistance. Although the concept of "one health" increases awareness of antibiotic resistance by offering holistic approaches to environmental, human, and animal health, bacterial resistance continues to pose a risk for the whole world. Therefore, it is important to conduct inventory studies on antibiotic resistance in marine areas.

Although the levels of antibiotic-resistant bacteria in aquatic areas fluctuate depending on geographical antibiotic exposure, aquatic environments pose a global threat to the spread of antibiotic resistance to a greater or lesser extent. Therefore, it is important to monitor the frequencies of antimicrobial resistance in areas subjected to different anthropogenic pressures, especially coastal areas, through long-term studies.

The coastal areas of Kınalıada were under anthropogenic pressure based on detecting high levels of antibiotic-resistant bacteria. This indicates that the region is at potential risk to human and ecosystem health due to the presence of antibiotic-resistant bacteria. Considering that the global climate change process negatively affects bacterial antibiotic resistance in aquatic environments, it is important to inventory bacterial antibiotic resistance in marine environments to mitigate climate resistance. This research highlights the effects of anthropogenic impacts on marine areas on the spread of antibiotics and the development and spread of antibiotic-resistant bacteria and genes. The data obtained in this study contribute to bacterial data on regional antibiotic resistance, but monitoring studies are needed to ensure that the data are continuously updated.

The high frequency of bacterial antibiotic resistance and multiple antibiotic resistance levels detected for the first time in the coastal area of Kınalıada in this study necessitate the monitoring of the region for studies to be compared with the entire Marmara Sea. The follow-up of these basic data with long-term monitoring studies will provide the necessary data for the development of measures in this regard.

Conflicts of Interest: The authors declare no conflicts of interest.

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

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