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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Antibiotic Resistance of Marine Bacteria on the Sediments of the Black Sea-Comparison of CLSI and EUCAST

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Abstract: Marine sediments are the important reservoirs of antibiotics and resistant genes. Antibiotic resistance becomes a global concern today and bacteria from marine sediments show resistance to multiple microbial agents. This study investigates antibiotic resistance of Gram-negative heterotrophic bacteria from the marine sediment of the Black Sea. The samples were gathered from various sampling locations on the marine environments of the Black Sea between May 2019 and February 2020. The VITEK 2 system was utilized to detect the MIC values against different antibiotics automatically. The MIC values were ranged from 0.12 µg/ml to 80 µg/ml. The highest resistance ratios among all antibiotics were detected as 90% in CLSI and 81% in EUCAST against cefazolin. In general, the resistance ratios were determined as 13.4% in CLSI and 26.1% in EUCAST. All isolates were detected as susceptible to meropenem, cefepime, ceftazidime, colistin, ertapenem, and piperacillin/tazobactam. MAR indexes were ranged between 0 and 0.45. MAR index were detected above or equal the value of 0.2 as 27% in CLSI and 81% in EUCAST among all isolates. This study shows that EUCAST may be better than CLSI in terms of showing the antibiotic exposure among natural isolates and the MAR indexes may be a useful tool for defining the environmental status. The results of this study indicate that antibiotic resistance may be a serious concern in sediments of the Black Sea.

Keywords: Antibiotic, bacteria, black sea, CLSI, EUCAST, sediment.

Karadeniz Sedimentlerinden Izole Edilen Deniz Bakterilerinin Antibiyotik Direnci-CLSI ve EUCAST Karşılaştırması

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Öz: Deniz sedimentleri, antibiyotiklerin ve dirençli genlerin önemli rezervuarlarıdır. Antibiyotik direnci günümüzde küresel bir sorun haline gelmektedir ve deniz sedimentlerinden gelen bakteriler, çoklu mikrobiyal ajanlara direnç göstermektedir. Bu çalışma, Karadeniz'in deniz sedimentlerinden izole edilen Gram-negatif heterotrofik bakterilerin antibiyotik direncini araştırmaktadır. Örnekler, Mayıs 2019 ile Şubat 2020 arasında Karadeniz'deki çeşitli örnekleme istasyonlarından toplanmıştır. VITEK 2 sistemi, farklı antibiyotiklere karşı MIC değerlerini otomatik olarak tespit etmek için kullanılmıştır. MİK değerleri 0,12 µg/ml ile 80 µg/ml arasında değişiklik göstermiştir. Tüm antibiyotikler arasında en yüksek direnç oranları sefazoline karşı CLSI'de %90 ve EUCAST'da %81 olarak tespit edilmiştir. Direnç oranları genel olarak CLSI'de %13,4, EUCAST'da ise %26,1 olarak belirlenmiştir. Tüm izolatların meropenem, sefepim, seftazidim, kolistin, ertapenem ve piperasilin/tazobaktama duyarlı olduğu tespit edilmiştir. MAR indeksleri 0 ile 0,45 arasında bulunmuştur. Tüm izolatlarda 0,2 değerinin üzerinde veya eşit olan MAR indeksi CLSI'de %27 ve EUCAST'ta %81 olarak tespit edilmiştir. Bu çalışma, doğal izolatlar arasında antibiyotik maruziyetini gösterme açısından EUCAST'ın CLSI'den daha iyi olabileceğini ve MAR indeksinin çevresel durumu tanımlamada yararlı bir araç olabileceğini belirtmektedir. Bu çalışmanın sonuçları, Karadeniz sedimentlerinde antibiyotik direncinin ciddi bir endişe kaynağı olabileceğini göstermektedir.

Anahtar kelimeler: Antibiyotik, bakteri, CLSI, EUCAST, karadeniz, sediment.

INTRODUCTION

Marine sediments are the significant reservoirs of various pollutants, antibiotics, and resistance genes. Bacteria from marine sediments may play a crucial role in the transfering of antibiotic resistance between different ecosystems around the world (Yang et al., 2013). Some bacterial species can become resistant to antibiotics by adapting to environmental conditions. Issues such as climate change may also increase the resistance of bacteria. Human activities and climate change accelerate the resistance of pathogens to antibiotics. The spread of pathogens carried by water and other factors may lead occurring of diseases in humans and other organisms (MacFadden et al., 2018; Cavicchioli et al., 2019). Today, bacterial species that have become resistant to antibiotics through natural selection are getting stronger by making the mechanism of action of drugs dysfunctional. The World Health Organization (WHO) has taken many initiatives in this regard. WHO has announced that active measures should be taken against danger of the antibiotics immediately. Rational use of antibiotics was advised in 2005 and all countries were invited to take precautions against antibiotic resistance. It is estimated that large numbers of people will die from antibiotic-resistant pathogens by 2050 (WHO, 2015).

As a result of antibiotic tests applied to bacteria of clinical origin that can cause various diseases and infections in hospitals, it was determined that these bacteria gained resistance to various types and doses of antibiotics (Ug & Ceylan, 2003; Mikolay et al., 2010; Lima de Silva et al.., 2012; Yamina et al., 2014; Sütterlin et al., 2018). Antibiotics cannot be completely absorbed in the human and animal digestive tract. These antibiotics are mixed into the environment and then into the sea through hospital wastes, wastewater, human and animal feces. According to the antibiotic types, these antibiotics mix with groundwater and seas (Schlusener & Bester, 2006; Kim et al., 2011; Matyar, 2012). These bacteria which can reach the seas in various ways can endanger fisheries and public health. Besides, it is known that the resistance of bacterial species increases due to antibiotics and chemicals used in fish farms (Akinbowale et al., 2007). Moreover, it has been reported that there is antibiotic pollution in rivers and streams (Miranda & Castillo, 1998; Pathak & Gobal, 2005; Özaktaş et al., 2012; Xu et al., 2019). It has been also reported in the publications that antibiotic-resistant bacteria reaching the marine environment have multiple plasmids that enable them to acquire a resistance mechanism and carry their genes by transferring them by conjugation (Thavasi et al., 2007).

Turkey ranks first in terms of antibiotic consumption. According to the Organisation for Economic Co-operation and Development, the antibiotic resistance ratio of Turkey in 2015 was reported as 35% which was the highest among the member states. This ratio was detected as seven times higher than the lowest ratio in the organization. The Ministry of Health in Turkey has set rules to decrease the usage of antibiotics such as ceftazidime, carbapenem, cefepime, glycopeptides, and piperacillin/tazobactam. In the comparison of antibiotic resistance against the eight most important antibiotic combinations between OECD and non-OECD countries in 2015, the country with the highest rates was India with 58%, while Turkey ranked 7th in the world. Turkey was the highest resistance rate among OECD countries (38.8%) and it took first place as the country with the most antibiotic drug use. Despite the 15-year hospital antibiotic restriction program, it was emphasized that health policies should be reviewed urgently and an effective prevention program should be implemented (Altunsoy et al., 2011; Isler et al., 2019). These antibiotics which reach the sea in various ways threaten public and ecosystem health. For this reason, it is essential to monitor the antibiotic resistance of the bacteria from marine environments constantly. Furthermore, bacterial activities on the marine ecosystem and marine sediments should be determined to develop measures against deterioration in the environment.

Previous researches have indicated that the Black Sea especially coastal areas is under the pressure of bacteriological pollution and antibiotic resistance (Altuğ et al., 2008; Terzi and İşler, 2019; Kalkan and Altuğ, 2020, Sabatino et al., 2020; Prekrasna et al., 2022). Several studies have also revealed that bacterial isolates obtain from fish farms and other species were resistant to various antibiotics (Durmaz et al., 2012; Üstünakın et al., 2015; Rakici et al., 2021; Kayış et al., 2021). Despite this, very few studies have focused on resistance of marine sediment bacteria in the Black Sea.

In this study, antibiotic resistances of Gram-negative heterotrophic bacteria from the marine sediment of the Black Sea against the commonly used hospital and veterinary acquired antibiotics were investigated. Resistance ratios were evaluated and compared based on different organizations' guidelines for antibiotic testing. In particular, the spread of antibiotic misuse which is still up to date has been interpreted and the risks it carries for human health and ecosystem functions in the marine ecosystem and especially in the marine sediments have been defined.

MATERIAL AND METHOD

Sampling location: The marine sediment samples were gathered from various sampling locations on marine environments of the Black Sea on a seasonal basis between May 2019, and February 2020 (Figure 1).

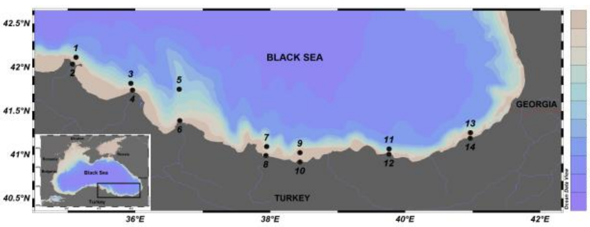

Figure 1. The locations of the stations in the Black Sea.

Ekman grab sampler was utilized to take samples from surface sediment layers (0-5 cm). Different kinds of sediments from various depths were analyzed (Figure 2).

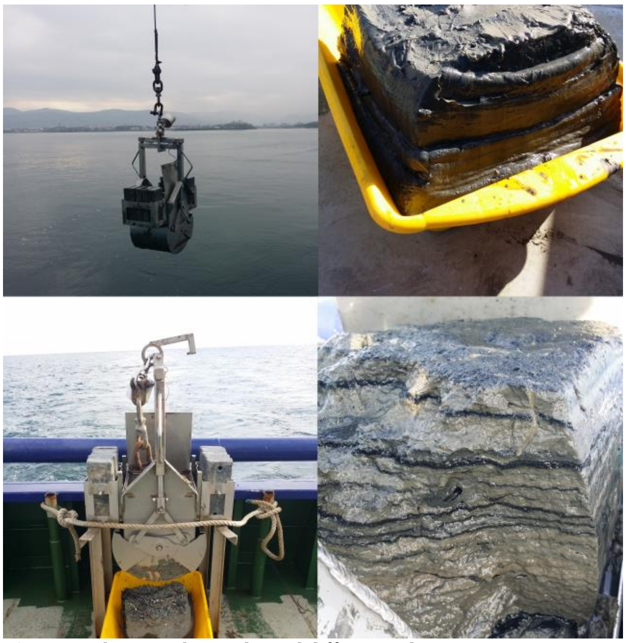

Figure 2. Ekman grab sampler and different sediment structures.

Eleven environmental isolates which were identified on the marine sediment samples of the Black Sea (as laboratory stocks) were tested against different antibiotics. Gram-negative environmental isolates used in this study were summarized by giving detailed information and accession numbers (contains coordinates, stations, etc.) located on the GenBank (Table 1).

Table 1. Tested Gram-negative environmental isolates and accession numbers.

No	Code	Matched bacteria species	Isolation area	Accession number
1	SK-6	*Acinetobacter calcoaceticus*	Ordu	OL839957
2	SK-7	*Serratia nematodiphila*	Rize	OL839958
3	SK-14	*Serratia marcescens*	Rize	OL839965
4	SK-15	*Pseudomonas monteilii*	Sinop	OL839966
5	SK-17	*Hafnia paralvei*	Samsun	OL839968
6	SK-18	*Pantoea agglomerans*	Ordu	OL839969
7	SK-19	*Pseudomonas moraviensis*	Samsun	OL839970
8	SK-20	*Pantoea agglomerans*	Ordu	OL839971
9	SK-24	*Pseudomonas moraviensis*	Samsun	OL839975
10	SK-36	*Achromobacter deleyi*	Giresun	OL839987
11	SK-38	*Leclercia adecarboxylata*	Trabzon	OL839989

Antibiotic susceptibility test (AST) and Multiple antibiotic resistance (MAR) index: The VITEK® 2 Antimicrobial Susceptibility Tests (AST) were used to determine antibiotic resistance of clinically important bacteria species isolated from marine sediments. AST-N325 cards were utilized to detect the Minimum Inhibitory Concentration (MIC) values and breakpoints. The VITEK® 2 Systems AST test is an automated test approach based on the MIC technique (MacLowry & Marsh, 1968; Gerlach 1974). The AST is essentially a compacted version of the microdilution method for MIC values (Barry, 1976).

64 microwells contain specified antimicrobials at various concentrations with dried culture medium on each AST card. Each AST card contains different types of antibiotic groups. Amikacin, ampicillin, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cefuroxime axetil, ciprofloxacin, colistin, ertapenem, gentamicin, meropenem, piperacillin/tazobactam, tigecycline, trimethoprim/sulfamethoxazole were tested depending on the species. Pure cultures depends on species were incubated on Trypticase Soy Agar with 5% Sheep Blood (TSAB) and Columbia Sheep Blood Agar (CBA) at 35°C to 37°C (aerobic and non-CO_2 conditions) for 18 to 24 hours. Pure cultures at between 0.5-0.63 McFarland turbidity concentration were inoculated to plastic test tubes filled with 3.0 ml of sterile saline solution (0.45% NaCl). 145 µl of this homogenous suspension was transferred in a second tube containing 3.0 ml of saline solution for manual dilution of AST-GN cards. The bacterial growth of each well was monitored after 24 hours automatically. The program measured the growth patterns of each organism in the existence of the antibiotic to the growth control well. Multiple factors depending on growing conditions were considered to assess the MIC and qualitative assessment. The MIC value for each antibiotic and species was calculated by defining the smallest concentration at which there was no growth (Weinstein et al., 2018). MIC values were interpreted based on the breakpoints declared by European Committee for Antimicrobial Susceptibility Testing (EUCAST) and Clinical & Laboratory Standards Institute (CLSI) (CLSI, 2018; EUCAST, 2021).

The multiple antibiotic resistance index (MAR) is effective in identifying the potential for contamination in high-risk environmental environments. MAR index values were calculated according to Krumperman (1983). Multiple antibiotic resistance index (MAR index) was measured according to the a/b formula. In this formula, “a” is the number of the resistant isolate against all antibiotics, and “b” is the number of all antibiotics tested on the isolate (Krumperman, 1983). The MAR index greater than 0.2 indicates a high risk of contamination, especially with fecal pollution from human-made wastes and animals, while the MAR index of less than 0.2 indicates a low risk of pollution of animal origin, where antibiotics are used very little or not at all (Krumperman, 1983; Matyar et al. 2008; Erdem et al., 2015).

RESULTS

MIC values of Gram-negative isolates against different antibiotic groups were summarized in Table 2. The MIC values were ranged from 0.12 µg/ml to 80 µg/ml. The highest MIC value was detected as 80 µg/ml against trimethoprim/sulfamethoxazole on SK-15 (*Pseudomonas monteilii*). The lowest MIC values were determined as 0.12 µg/ml against cefepime, ceftazidime, and ertapenem on different strains. 38 antibiotic tests (20%) in a total of 187 antibiotic tests were not applicable to some of the isolates.

Table 2. MIC scores (µg/ml) against to various antibiotics of Gram-negative bacteria isolates on the marine sediments of the Black Sea.

No	Antibiotics	SK-6	SK-7	SK-14	SK-15	SK-17	SK-18	SK-19	SK-20	SK-24	SK-36	SK-38
1	Amikacin	<=2	<=2	<=2	4	<=2	<=2	4	<=2	<=2	4	<=2
2	Ampicillin	-	>16	>16	-	<=2	16	-	16	-	-	<=2
3	Cefazolin	>32	>32	>32	>32	<=4	32	>32	32	>32	32	<=4
4	Cefepime	8	<=0.12	<=0.12	2	<=0.12	<=0.12	2	<=0.12	2	8	<=0.12
5	Cefoxitin	-	16	>32	-	<=4	8	-	16	-	-	<=4
6	Ceftazidime	8	0.25	0.25	2	0.25	<=0.12	2	<=0.12	2	0.25	<=0.12
7	Ceftriaxone	16	1	<=0.25	-	<=0.25	1	-	0.5	-	-	<=0.25
8	Cefuroxime	-	>32	>32	-	<=1	16	-	16	-	-	<=1
9	Cefuroxime Axetil	-	>32	>32	-	<=1	16	-	16	-	-	<=1
10	Ciprofloxacin	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	2	<=0.25
11	Colistin	<=0.5	-	-	-	-	-	-	-	-	<=0.5	-
12	Ertapenem	-	<=0.12	<=0.12	-	<=0.12	<=0.12	-	<=0.12	-	-	<=0.12
13	Gentamicin	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
14	Meropenem	<=0.25	<=0.25	<=0.25	1	<=0.25	<=0.25	0.5	<=0.25	1	<=0.25	<=0.25
15	Piperacillin/Tazobactam	8	<=4	<=4	8	<=4	<=4	8	<=4	8	<=4	<=4
16	Tigecycline	<=0.5	1	1	2	<=0.5	<=0.5	1	<=0.5	1	<=0.5	<=0.5
17	Trimethoprim/Sulfamethoxazole	<=20	<=20	<=20	80	<=20	<=20	<=20	<=20	40	<=20	<=20

SK-6: (*Acinetobacter calcoaceticus*), SK-7:(*Serratia nematodiphila*), SK-14: (*Serratia marcescens*) SK-15: (*Pseudomonas monteilii*), SK-17: (*Hafnia paralvei*), SK-18: (*Pantoea agglomerans*), SK-19: (*Pseudomonas moraviensis*), SK-20: (*Pantoea agglomerans*), SK-24: (*Pseudomonas moraviensis*), SK-36: (*Achromobacter deleyi*), SK-38: (*Leclercia adecarboxylata*).

Interpretation of MIC values regarding CLSI and EUCAST, and calculated MAR indexes regarding the number of total and resistant strains against antibiotics were summarized in Table 3. The highest resistance ratios among all antibiotics were detected as 90% in CLSI and 81% in EUCAST against cefazolin. All isolates except SK-38 (*Leclercia adecarboxylata*) and SK-17 (*Hafnia paralvei*) which were determined as susceptible and increased exposure were resistant to cefazolin. In general, the resistance ratios general were 13.4% (20 tests) in CLSI and 26.1% (39 tests) in EUCAST among 149 applicable tests for each antimicrobial susceptibility testing organization.

The lowest resistance ratios among all antibiotics were measured as 0% both in CLSI and EUCAST against meropenem, cefepime, ceftazidime, colistin, ertapenem, and piperacillin/tazobactam. All isolates were detected as susceptible to meropenem. In general, the susceptibility ratios were 80.5% (120 tests) in CLSI and 51.6% (77 tests) in EUCAST among 149 applicable tests for each antimicrobial susceptibility testing guideline. 9 tests (6%) were identified as intermediate in CLSI. 19 tests (12.7%) were interpreted as susceptible, increased exposure (I*) in EUCAST.

Table 3. Calculated MAR indexes and Interpretation of MIC values regarding CLSI and EUCAST.

		SK-6		SK-7		SK-14		SK-15		SK-17		SK-18		SK-19		SK-20		SK-24		SK-36		SK-38		RPAİ (%)	
No	**Antibiotics**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**
1	Amikacin	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	0	27
2	Ampicillin	-	-	R	R	R	R	-	-	R	S	I	R	-	-	I	R	-	-	-	-	S	S	50	66
3	Cefazolin	R	R	R	R	R	R	R	R	R	I*	R	R	R	R	R	R	R	R	R	R	S	I*	90	81
4	Cefepime	S	I*	S	S	S	S	S	I*	S	S	S	S	S	I*	S	S	S	I*	S	I*	S	S	0	0
5	Cefoxitin	-	-	I	*	R	*	-	-	S	*	S	*	-	-	I	*	-	-	-	-	S	*	16	0
6	Ceftazidime	S	I*	S	S	S	S	S	I*	S	S	S	S	S	I*	S	S	S	I*	S	S	S	S	0	0
7	Ceftriaxone	R	R	S	S	S	S	-	-	S	S	S	S	-	-	S	S	-	-	-	-	S	S	14	14
8	Cefuroxime	-	-	R	R	R	R	-	-	S	I*	I	R	-	-	I	R	-	-	-	-	S	I*	33	66
9	Cefuroxime Axetil	-	-	R	*	R	*	-	-	S	S	I	R	-	-	I	R	-	-	-	-	S	S	33	33
10	Ciprofloxacin	S	S	S	S	S	S	S	I*	S	S	S	S	S	I*	S	S	S	I*	I	R	S	S	0	9
11	Colistin	S	S	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	S	*	-	-	0	0
12	Ertapenem	-	-	S	S	S	S	-	-	S	S	S	S	-	-	S	S	-	-	-	-	S	S	0	0
13	Gentamicin	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	S	S	R	S	R	S	S	0	54
14	Meropenem	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0
15	Piperacillin/Tazobactam	S	S	S	S	S	S	S	I*	S	S	S	S	S	I*	S	S	S	I*	S	S	S	S	0	0
16	Tigecycline	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	S	S	R	S	S	S	S	0	45
17	Trimethoprim/Sulfamethoxazole	S	R	S	*	S	*	R	*	S	R	S	S	S	*	S	S	S	*	S	R	S	R	9	45
	MAR Index	0.16	0.25	0.25	0.35	0.31	0.35	0.20	0.30	0.12	0.06	0.06	0.25	0.10	0.30	0.06	0.25	0.10	0.30	0.09	0.45	0.00	0.06		

S=Susceptible; I=Intermediate (CLSI); I*= Susceptible, increased exposure (EUCAST); R=Resistant; –=not applicable; *=Categorising the susceptibility of the organism is not possible. There is no approved method and breakpoints have not been determined (EUCAST)

SK-6: (*Acinetobacter calcoaceticus*), SK-7:(*Serratia nematodiphila*), SK-14: (*Serratia marcescens*) SK-15: (*Pseudomonas monteilii*), SK-17: (*Hafnia paralvei*), SK-18: (*Pantoea agglomerans*), SK-19: (*Pseudomonas moraviensis*), SK-20: (*Pantoea agglomerans*), SK-24: (*Pseudomonas moraviensis*), SK-36: (*Achromobacter deleyi*), SK-38: (*Leclercia adecarboxylata*), RPAİ: Resistance percentageFor all isolates

MAR indexes were ranged between 0 and 0.45 regarding isolates, antibiotics, and interpretation guidelines. MAR indexes were detected above or equal the value of 0.2 among 3 isolates (27%) in CLSI and 9 isolates (81%) in EUCAST. The highest MAR index was measured as 0.45 in SK-36 (*Achromobacter deleyi*) regarding EUCAST and The lowest MAR index was calculated as 0.00 in SK-38 (*Leclercia adecarboxylata*) regarding CLSI. The MAR indexes were computed higher in EUCAST than CLSI except SK-17 (*Hafnia paralvei*).

DISCUSSION AND CONCLUSION

Interestingly, antibiotic resistance researches conducted on sediments of the Black Sea are limited specifically in the research area. Recent evidence suggests that the Black Sea contains effective antibiotic resistance genes especially in the water columns. It is also reported that resistance genes abundance was higher in deep layers below 100 meters surprisingly (Sabatino et al., 2020). According to Özcan et al., (2013), marine actinomycetes isolates are more active than soil isolates in producing antimicrobial compounds. In the study of Gul-Seker and Mater, (2009), isolates from the Bosphorus entrance of The Black Sea water column show multiple antibiotic resistance to several agents up to 5 antibiotics such as trimethoprim-sulfamethoxazole. Erdem et al., (2017) reported isolates from Kilyon coasts in the Black Sea were detected as resistant to penicillin (93%) and ampicillin (48%) while susceptible to gentamicin (90%) and amikacin (90%). Ture et al., (2018) reported that sediment bacteria gain noticeable resistance abilities because of fish farming in the Black Sea. Akkan and Mutlu (2016) revealed that isolates from seawater of Giresun Coast in the Black Sea show resistance to cefazolin (46.50%), amikacin (41.50%), cefuroxime (35.50%), and ampicillin (15.50%). They also reported that the MAR indexes above 0.2 were higher in 91% of all isolates. According to the study by Kimiran-Erdem et al., (2007), bacterial isolates from the Bosphorus and entrance of The Black Sea have resistance to amikacin (25%), ampicillin (6%), and gentamycin (2%). Similarly, the current study found that bacteria from the marine sediments of the Black Sea show resistance to commonly used antibiotics such as cefazolin and ampicillin although the current study was unable to detect whether the resistance is natural or acquired. However, the findings of this study would seem to suggest that the same species such as SK-18, SK-20 (*Pantoea agglomerans*), and SK-19, 24 (*Pseudomonas moraviensis*) show different resistance and MIC values against to same antibiotics may be a piece of evidence that antibiotic resistance is not natural, but acquired later. Taken together, these findings suggest that there may be a concerning antibiotic exposure and contamination in marine sediments of the Black Sea.

On the other hand, Akkan’s (2017) findings show that isolates from the Batlama Creek were resistant to ampicillin (75%), amikacin (34%), and cefazolin (33%). Akkan (2017) also revealed high MAR indexes (in 77% of all isolates) which indicates antibiotic pollution in the environment. Işık and Akkan (2021) reported that isolates from the Gelevera Creek show resistance to cefazolin (69.6%), cefuroxime (59.4%), ampicillin (46%), and amikacin (21.7%). Their MAR index ratio (80%) reveals that the Gelevera Creek which is discharge to the Black Sea carries tremendously resistant bacteria against common antibiotics. Previous studies have also demonstrated that bacteria from fish species also show resistance to ampicillin, sulfamethoxazole, trimethoprim (Kayış et al., 2009; Capkin et al., 2015; Ture & Alp, 2016). Rakici et al., (2021) reported that Gram-negative bacteria isolated on frog species from the Eastern Black Sea are resistant to ampicillin and cefazolin. Therefore, it can be assumed that the creeks, streams, rivers, and organisms also may be important antibiotic transporters to the sediments of the Black Sea. It could conceivably be hypothesized that resistance in sediment may be even higher than seawater, coastal areas, and rivers regards the accumulation and persistence of antibiotics without sufficient degradation.

Multiple antibiotic resistance is a concerning issue for human health. MAR index is an important index to indicate antibiotic exposure in the source of animals and humans. In the research of Gul-Seker and Mater (2009), MAR indexes were found between 0.250 and 0.625. Erdem et al., (2017) revealed that high MAR values show antibiotic contamination because of the human effects. Likewise, in the current study, the MAR indexes showed high-risk fecal contamination from human-made wastes and animals. It can thus be suggested that the MAR index may be a useful tool for defining the resistance levels as well as the environmental status for the anthropogenic impacts.

In recent years, there has been an increasing interest in comparing CLSI and EUCAST standarts (Mendes et al., 2010; Sader et al., 2021). While CLSI standards were used in Turkey before, institutions and organizations in Turkey have started to use EUCAST standards with the widespread acceptance of EUCAST standards with the advice of the European Union. In this context, the values found in the EUCAST limit tables have gained importance. It has become necessary to determine the MIC values of bacteria to understand whether there is a dangerous situation for the ecosystem and public health in terms of the effects of antibiotics (Gur et al., 2016). Since most of these antibiotics originate from hospitals, it is important to detect possible pollution risks from hospitals to the sea. It is known that Turkey is at the top of the misuse of antibiotics and antibiotic misusage is increasing dramatically. Resistant sediment bacteria against common antibiotics may infect people and cause serious illness in long term. Even new generation antibiotics may become ineffective against these wild and evolved bacteria that have gained resistance and pose serious problems for human health. The results of this study also show that EUCAST may be better than CLSI in terms of showing the antibiotic exposure not only in clinical and but also in environmental isolates and marine sediments as well.

Super-bacteria (superbugs) are defined as bacteria that are almost resistant to all known antibiotics and cannot be killed by antibiotics, and their importance in antibiotic resistance approaches is increasing day by day (Arias & Murray, 2009; Duarte-Neto, 2019). Super-bacteria which have been found to be resistant to β-lactam and all its derivative antibiotics started to cause panic by posing a danger to public health with the reporting of new clinical cases since 2010 (Chung & Lee, 2011; Jacome & Gonzales-Zubiate, 2019). The current research was not specifically designed to evaluate super-bacteria skills. However, the evidence from this study suggests that antibiotic-bacteria in sediments of the Black Sea may evolve to super-bacteria with the increase of excessive antibiotic exposure in time and damage human health and marine ecosystems.

Although the One Health approach is a concept that has recently started to be used today its history dates back to ancient times. One Health emphasizes the importance of multidisciplinary work to create optimal health for the public and the environment at the national and international level.

Emerging epidemics have led us to use interdisciplinary sciences for challenging health problems. The One Health has come popular because of intersectoral and interdisciplinary approaches which give extra advantages. An important point in the single health approach is antibiotic misuse and resistance (Léger et al., 2018; Rüegg et al., 2018). The One Health perspective is advised by the scientists and several communities to tackle emerging problems such as antibiotic resistance. Approximately 700,000 people in the world die annually due to antibiotic-resistant pathogens. It is estimated that this number will reach ten million people by 2050 and the financial loss will be 100 trillion dollars. In this case, the damage of antibiotic resistance will leave cancer behind. It has long been known that antibiotic resources are wasted. It is stated that urgent action should be taken to reverse current trends in antibiotic resistance with a single health approach (Zinsstag et al., 2009; Collignon, 2015; Robinson et al., 2016; Kingsley & Taylor, 2017; European Commission, 2017; McEwen & Collignon, 2018). The results of this research suggest the idea that the marine environments and especially sediments may play a crucial role in terms of showing antibiotic resistance and exposure in the aspect of the One Health concept.

The results of this study has indicated that antibiotic resistance may be a serious concern in sediments of the Black Sea. More research is required to determine the impact of resistant marine sediment bacteria on One Health approach. Further investigation using shotgun metagenomic sequencing to define antibiotic resistance in better aspects is strongly recommended. Also, future research needs to examine more closely the links and similarities in terms of resistance between clinical human pathogens and marine sediment bacteria.

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