Research Article

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Antibiotic Resistance and Phylogeny of Bacterial Isolates with Biogeochemical Analysis from Sediments of Eastern Mediterranean Sea

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Abstract

***Background**: Bacteria in marine environments show different diversity and resistance patterns. In this study, the phylogeny and antibiotic susceptibility levels of bacterial strains isolated from sediments of Eastern Mediterranean Sea (0–1235 m depths) were analyzed in association with geochemical parameters of sediments.*

***Methods**: Bacterial isolation was performed and totally 185 isolate whose 16S rRNA gene sequences were deposited into NCBI Gen Bank were assayed with disk diffusion method using eleven antibiotics. Statistical comparison was performed for susceptibility levels of strains and geochemical parameters of stations as grain size and carbon, nitrogen, phosphorus contents of sediment samples.*

***Results and Conclusion**: The highest resistance was mostly to amikacin and ceftazidime. While the Bacillus strains with the highest diversity had the highest resistance, the genera Planococcus, Marinobacter, Psychrobacter and Vibrio were susceptible to all antibiotics and even the genera Halobacillus, Fictibacillus, Lysinibacillus, Salinimonas, Photobacterium, Planococcus, Psychrobacter and Vibrio had no intermediate level. The geochemical contents of the sediments and susceptibility levels of the bacterial isolates were not statistically correlated but there was positive correlation between grain size and resistance. Due to the influence of terrestrial and anthropogenic factors, the shallowest stations had the highest resistance and were separated from deep-basins in correlation analysis.*

Keywords: Antibiotic susceptibility levels, Bacterial diversity, 16S rRNA genes, Sediments, Eastern Mediterranean Sea.

Introduction

According to combination of marine bacterial 16S rRNA database, *Proteobacteria*, especially *Alphaproteobacteria* and then *Gammaproteobacteria* are dominant in overall marine environment and inverse is valid for culture-dependent studies [1-3]. In shallow and deep sediments, *Gammaproteobacteria* and *Firmicutes* (the genera *Bacillus* and *Clostridium*) become dominant but *Actinobacteria* form a smaller portion and occur in the photic zone and also sediments [4-6].

Antibiotic resistance is already present in natural environments but antibiotics used in medicine, livestock and fishfarms may either end up in soil, sediment, ground water or seawater [7-9]. For aquatic environment, the studies especially beneath fish farms have reported not only the changes in bacterial communities, widespread antibiotic resistance and the increase of the multiple resistance but also the high loads of antibiotics in the sediments [7-12].

Accumulation of indigenous bacteria resistant to several antimicrobial agents were highlighted in coastal area, especially near shore sediments and sandy beaches in addition to high multiple antibiotic resistance (MAR) index [13-20]. In spite of limited number of studies at Turkish coastal environment, similar results have been reported for the coasts from Istanbul to Canakkale in Marmara Sea, and from Canakkale to Iskenderun in eastern Aegean Sea and North Levantin Sea, with high MAR index for coastal seawaters and sediments [20-24].

Due to the scarcity of research about bacterial phylogeny and antimicrobial resistance in marine environments, in this study, the phylogenetic and antimicrobial analysis of bacteria isolated from sediments of coastal zone and deep-basins in Eastern Mediterranean Sea were aimed.

Methodology

Sediment Sampling and Analysis

In this study, sediment samples were taken from totally 24 stations with 0-1235 m depths of Eastern Mediterranean Sea for both bacterial isolation and sediment analysis (Figure 1). The sediment samples were collected into sterile plastic bags, 40 ml glass containers and sterile plastic cores for different processes and kept at –20ºC till the analysis.

The particle size of the sediments was determined by the sieve

analysis and the hydrometer method for the larger and the finer particles, respectively according to standard test method for particle size analysis of soils D 422-63 issued by American Society for Testing and Materials [25].

Total and organic carbon and nitrogen contents (TC and TOC, TN and TON, respectively) were obtained using Carlo Erba NC2500 model CHN analyzer, on the other hand, total and organic phosphorus contents (TP and TOP, respectively) were measured spectrophotometrically [26].

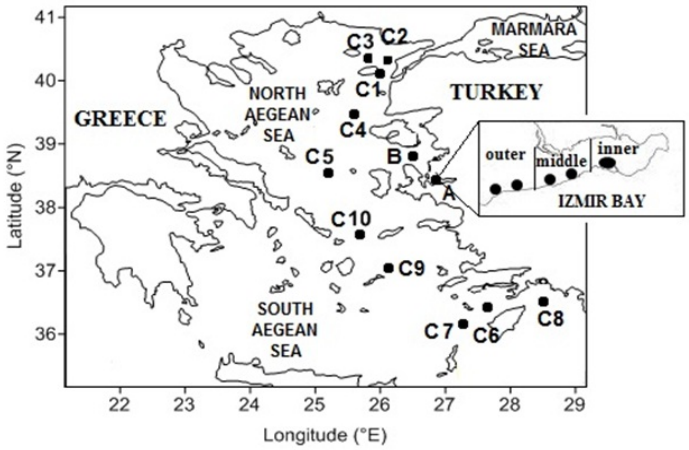

Figure 1: Stations A (9 stations), B (5 stations), and C1–C10 stations in Eastern Mediterranean Sea.

Bacterial Isolation and Physiological Analysis

Isolation of bacteria was achieved using seven different sediment processing methods and seven isolation media prepared with sterile seawater. The isolation media consisted of the following: M1, 18 g agar, 10 g starch, 4 g yeast extract, 2 g peptone, 1 liter sterile seawater; M2, 18 g agar, 1 g starch, 0.4 g yeast extract, 0.2 g peptone, 1 liter sterile seawater; M3, 18 g agar, 2.5 g starch, 1 g yeast extract, 0.5 g peptone, 750 ml sterile seawater, 250 ml distilled water; M4, 18 g agar, 1 liter sterile seawater; M5, 18 g agar, 750 ml sterile seawater, 250 ml distilled water; M6 (DifcoTM marine agar), 55 gr medium, 1 liter distilled water; M7 (DifcoTM actinomycete isolation agar, modified), 22 g medium, 5 ml glycerol, 500 ml sterile sea water and 500 ml distilled water. The isolation media M1 and M7 were used with or without six different antibiotics as cycloheximide (100 µg/ml), nystatin (50 µg/ml), polymixin B sulfate (5 µg/ml), rifampin (5 µg/ml), kanamycin sulfate (5 µg/ml), novobiocin (25 µg/ml).

Seven different sediment processing methods were performed. In the first processing method (a), 10 ml wet sediment sample were dried overnight and then 0.5 g dry sediment was aseptically spread in circular fashion onto the agar media [27]. In the dry spot method (b) dry sediment was taken with sterile sponge and put clockwise on the agar media. In the third method (c), 1 ml wet sediment was diluted with sterile seawater (1:4) and then heated for 6 min at 55°C. After vortexing for 30 s, 75-100 µl was spread aseptically onto agar-based isolation media [28]. In the fourth method (d), wet sediment was heated for 15 min at 70°C and then spread aseptically on the agar surface in a circular fashion [29]. In the fifth method (e), wet sediment was kept for 30 sec under UV and then spread aseptically in a circular fashion onto the agar media [30]. In the sixth method (f) 1 ml wet sediment sample was diluted with sterile seawater (1:1, 1:10 and 1:100) and then vortexed for 30 s. Then, 75–100 µl was spread aseptically onto agar-based isolation media. In the seventh method (g) without processing, wet sediment sample was aseptically spread onto agar-based isolation media.

The plates containing M6 medium were incubated at 20–22°C for 2-3 days and the rest at 26–28°C up to 2 months. The colonies selected according to colony morphology were sub cultured on M1, M6 or M7 media. Then, the isolates were cryopreserved with 50% glycerol at –20ºC.

Nuclear DNA Extraction and 16S rRNA Amplification

Genomic DNA of isolated bacteria was extracted with a commercial kit (Invitrogen, Carlsbad, CA) according to the user's manual for Gram-positive bacterial cell lysate.

The 16S rRNA genes were amplified from genomic DNA by PCR using the universal primer pairs of FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RC1492 (5′-TACGGCTACCTTGTTACGACTT-3′) and also the pairs of 63f (5′-CAGGCCTAACACATGCAAGTC-3′) and 1387r (5′-GGGCGGWGTGTACAAGGC-3′). The 50µl PCR mixture contained 20 to 50 ng of DNA, One Taq Quik-Load 2X Master mix (New England Bio labs, Inc. Beverly, MA), 10 pmol of each primer (Fermentas, Thermo Fisher Scientific, Waltham, MA), and 10 mM deoxynucleoside triphosphate mixture (Fermentas, Thermo Fisher Scientific, Waltham, MA). The PCR program consisted of 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min followed by a final extension step at 72°C for 7 min. Amplification products were examined by agarose gel electrophoresis.

Sequencing and Phylogenetic Analysis

Sequencing service was taken from Gene Research and Technology (RefGen, Turkey). For the phylogeny, all nucleotide sequences were analyzed using Geneious (version 6.1; Biomatters Ltd., NZ) and compared within the NCBI database (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis was performed using Mega with 1000 bootstrap neighbor-joining method [31].

All those partial 16S rRNA gene sequences have been deposited into GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/index.html) under the accession numbers KC815705–KC815847 and KF366670–KF366711.

Antibiotic Sensitivity Assay

Antibiotic tests were performed with disc diffusion method according to Performance Standards for Antimicrobial Disk Susceptibility Tests issued by Clinical and Laboratory Standards Institute [32]. Eleven antimicrobial agents representing different

classes of antibiotics were used as amikacin 30 µg (AN), ampicillin 10 µg (AM), chloramphenicol 30 µg (C), ceftazidime 30 µg (CAZ), cefotaxime 30 µg (CTX), ertapenem 10 µg (ETP), gentamicin 10 µg (GM), kanamycin 30 µg (K), nalidixic acid 30 µg (NA), trimethoprim/sulfamethoxazole 1.25µg/23.75µg (SXT), tetracycline 30 µg (TE).

Results

Sediment Parameters

In the study area, the grain sizes decreased with depth and stations A at intertidal zone of Izmir Bay had the largest particle size as from sandy to sandy gravel distribution. Ai stations at the inner bay (A1–A5) had finer particles compared to Aj stations in the middle and outer bays (A6 – A9). For B stations, approximately 100–150 m depths (Bi stations as B1, B3 and B5) were sandy while approximately 200 m depths (Bj stations as B2 and B4) were sand-silt-clay. C stations, on the other hand, had the finest particles as clayey silt, except C1 and C4 which were sandy at 72 m and silty sand at 207 m depth, respectively among other C stations (416–1235 m depths).

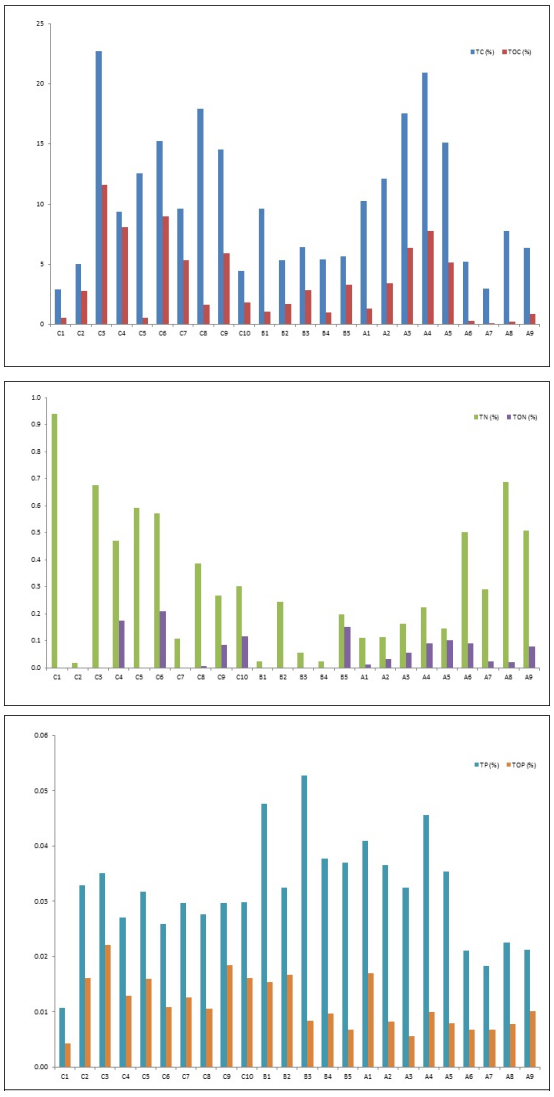

Figure 2: Chemical contents of sediments for A, B, and C stations in Eastern Mediterranean Sea. (TC: total carbon; TOC: total organic carbon; TN: total nitrogen; TON: total organic nitrogen; TP: total phosphorus; TOP: total organic phosphorus).

Chemical contents of sediments showed variation among stations A, B and C as reflecting the environmental and geographical differantiation (Figure 2). Similar to grain size results, separation of B stations from A and C stations, and Ai stations from Aj stations and even C1 station from C2 and C3 stations located very close to each other at the upper north Aegean Sea (Figure 1) were clearly seen in Figure 2 for total and organic carbon, nitrogen and phosphorus contents of the sediments.

While TC percent ranges were 2.97–20.94, 5.19–9.62 and 2.92–22.70, TOC percentages ranged as 0.12–7.77, 0.24–3.36, 0.56–11.59 for A, B and C stations, respectively. TN and TON, on the other hand, changed between 0.02% and 0.94 %, from below detection limit to 0.21%, respectively for the study area. Phosphorus contents (0.011–0.053 and 0.001–0.022 for TP and TOP percentages, respectively) did not vary as much as nitrogen did among all stations (Figure 2) indicating the nitrogen playing much more dynamic role in sediments.

Phylogenetic Diversity

According to 16S rRNA gene sequences of totally 185 isolates from sediments of Eastern Mediterranean Sea, a highly diverse phylum *Firmicutes* was obtained in addition to the phyla *Actinobacteria* and *Gammaproteobacteria* with cultivation-based methods (Figure 3).

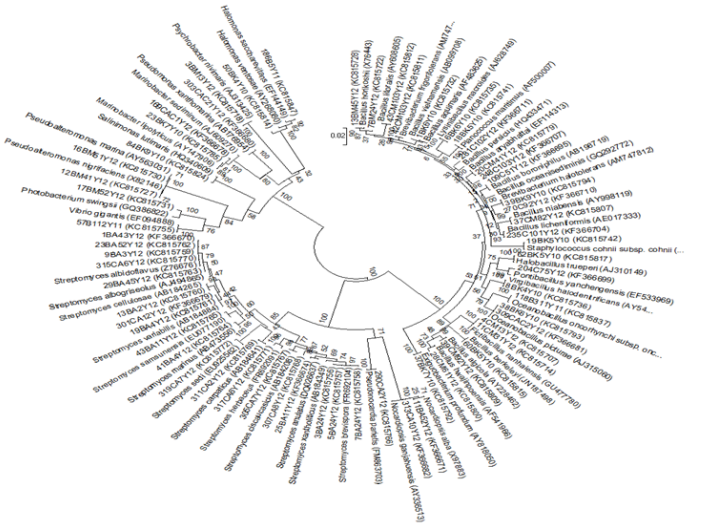

Figure 3: For phyla *Firmicutes, Actinobacteria* and *Gammaproteobacteria*, neighbor-joining distance tree was constructed using 16S rRNA gene sequences. GenBank accession numbers were given in parentheses. Bootstrap values calculated from 1000 re-samplings were in percentage.

A highly diverse clade with members belonging to the order *Bacillales* sharing a high phylogenetic affiliation with mostly *Bacillus species* in addition to the genera *Exiguobacterium, Fictibacillus, Halobacillus, Lysinibacillus, Oceanobacillus, Planococcus, Pontibacillus, Staphylococcus* and *Virgibacillus*

was isolated (Figure 3). For the phylum *Gammaproteobacteria*, 8 different genera as *Halomonas, Marinobacter, Psychrobacter, Pseudoalteromonas, Pseudomonas, Photobacterium, Salinimonas* and *Vibrio* were obtained (Figure 3). Furthermore, the actinomycete strains were found as closely related to the genera *Nocardiopsis, Pseudonocardia* and *Streptomyces* (Figure 3).

The *Halomonas* strain and the *Salinimonas* strain isolated from the stations A and the *Pontibacillus* strain from station C7 have a high probability of representing new taxa due to pairwise similarity < 98 % with their nearest type strains. When a limit of 98.5 to 99 % was considered, as revised and proposed by the number of new taxa increases such as the *Bacillus*, *Halomonas, Photobacterium* and *Streptomyces* strains isolated from stations B, C3, C4, C6, C8–C10 (9% of total isolates) [33].

The *Actinobacteria* and *Gammaproteobacteria* classes and the genera (belonging to the order *Bacillales*) other than the genus *Bacillus* were mainly isolated from stations A and B, additionally few from stations C1–C3 and C6–C8 (Figure 4). The shallowest sediments belonging to stations A had the highest phylogenetic diversity in higher taxa in addition to even higher diversity in stations Aj than Ai as illustrated in Figure 4.

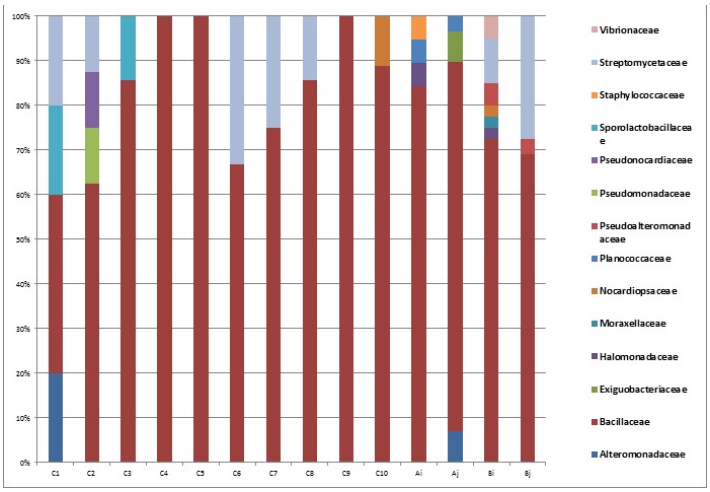

Figure 4: The percentages of nearest type families isolated from stations in Eastern Mediterranean Sea.

In contrast, the deep and oligotrophic stations C4, C5, C9, C10 in North Aegean Sea and C6–C8 in South Aegean Sea had higher diversity in lower taxa (Figure 4). However, compared to those stations, diversity increased at the family level for stations C1–C3, especially station C1 in North Aegean Sea. On the other hand, stations B showed the highest bacterial diversity in both lower and higher taxa, in addition the diversity of *Gammaproteobacteria* were higher in Bi and *Actinobacteria* were higher in Bj (Figure 4).

The cluster analysis based on the bacterial community composition at the genus level also revealed the phylogenetic differences for stations as illustrated in the dendogram given in Figure 5 supporting their geochemical and locational variations (Figure 2 and 4).

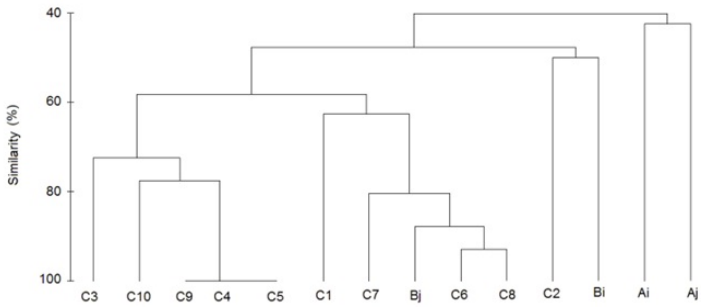

Figure 5: Cluster analysis dendogram based on the comparison of bacterial community at the genus level from different sediment samples.

Antibiotic Resistance

Compared to the C stations, much higher resistance were obtained from stations A and B (69 % and 50 %, respectively) in addition to higher MAR index (19 % and 14 %, respectively) as seen in Figure 6. On the other hand, susceptibility levels in deep-basins were 40–67 % whereas stations C1 and C3 had the highest resistance values (67 % and 57 %, respectively) among C stations but multiple resistance was seen only in stations C1–C4 and C8 (Figure 6).

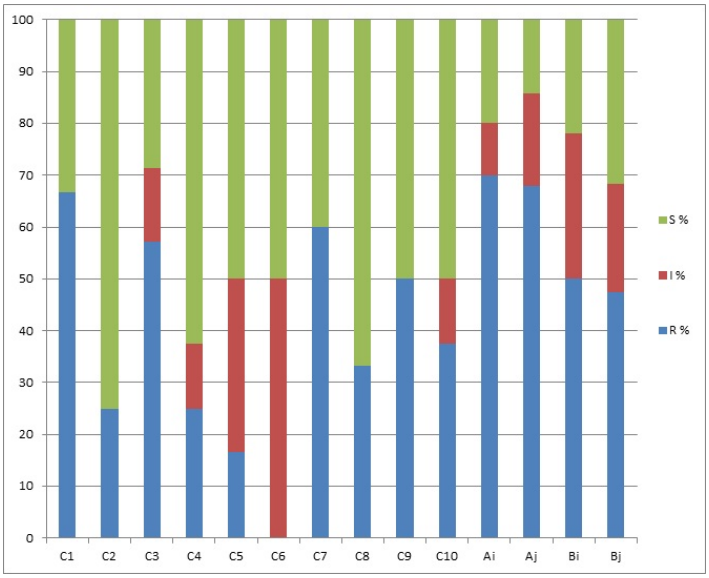

Figure 6: Resistance (R), intermediate (I) and susceptibility (S) percentages for isolates from sediments of Eastern Mediterranean Sea.

While the genera *Planococcus, Marinobacter, Physchobacter* and *Vibrio* were susceptible to all antibiotics, there was no intermediate level for the genera *Halobacillus, Fictibacillus, Lysinibacillus, Salinimonas, Photobacterium, Planococcus, Physchobacter* and *Vibrio*.

The highest resistance were found to antibiotics AN and then CAZ with totally 45 (35 % of total strains) and 28 strains (22 %), respectively (Figure 7).

The intermediate levels were also highest to AN (18 strains) and then CTX (14 strains). However, there was neither intermediate to ETP nor resistance to C.

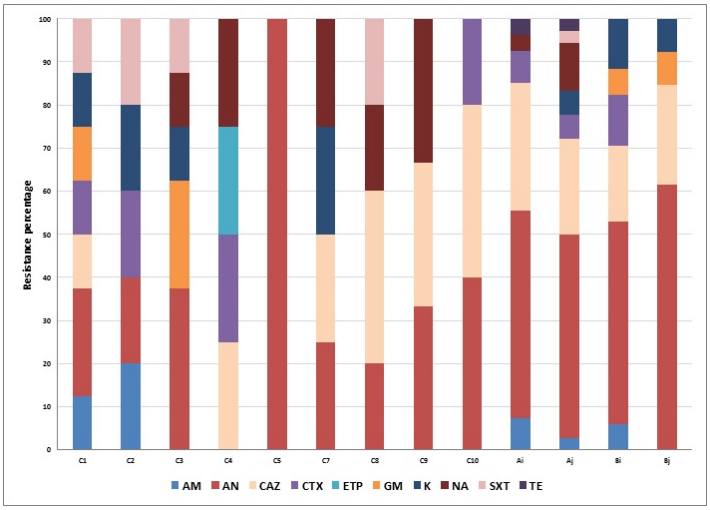

Figure 7: For the order *Bacillales*, the classes *Actinobacteria* and *Gammaproteobacteria*, neighbor. Amikacin 30 µg (AN), ampicillin 10 µg (AM), chloramphenicol 30 µg (C), ceftazidime 30 µg (CAZ), cefotaxime 30 µg (CTX), ertapenem 10 µg (ETP), gentamicin 10 µg (GM), kanamycin 30 µg (K), nalidixic acid 30 µg (NA), trimethoprim/sulfamethoxazole 1.25µg/23.75µg (SXT), tetracycline 30 µg (TE).

According to the statistical analysis of Pearson correlation, a positive correlation was seen between antibiotic resistance and sediment types of stations A, B and C ($p<0.05$). This expected result supported the fact that that coastal zones affected by terrestrial and anthropogenic inputs had higher resistance compared to deep-basins. There was also positive correlation between bacterial numbers (in both lower and higher taxa) and resistance and even MAR index ($p<0.05$).

Discussion

The geochemical composition of the study area was generally in the range of Aegean Sea. It was previously shown that environmental parameters together with geographical differences influenced the bacterial community composition in sediments of Eastern Mediterranean Sea [4,34-36]. For instance, in a previous study, cluster analysis of bacterial compositions supported the environmental and geographical differences and especially gave the north-south latitudinal separation for bacterial diversity in sediments of Eastern Mediterranean Sea [35]. Furthermore, in another study, when 16S rRNA clone compositions for North East Pacific and Eastern Mediterranean sediments were compared, hierarchical cluster analysis again provided the geographical separation [4]. Similarly, in the present study, the geochemical differences in addition to the variations of the geomorphologic, hydrographic and trophic states reflected on the phylogenetic diversity as supported in distribution and hierarchical cluster analysis of the isolates and also on the antibiotic resistance distribution for stations.

In a previous study of Eastern Mediterranean Sea, the relation between antibiotic resistance and geographical differences was given as higher resistance in coastal areas of Syria than Turkey and Lebanon (48 %, 38 % and 31 % of total isolates, respectively) [23]. Similarly, compared to the deep basins of the study area in Eastern Meaditerranean Sea, the highest resistance and the highest phylogenetic diversity in higher taxa were found for the isolates from coastal sediments of Izmir Bay (stations A) due to the dynamic environmental factors as continuous terrestrial and anthropogenic effects i.e. river and sewage input increasing the eutrophication. Furthermore, the higher influence of those inputs on the inner bay (stations Ai) was clearly seen as biogeochemical separation from the middle and outer bays (stations Aj). In contrast, the deep and oligotrophic C stations in middle north and lower south parts of the study area had higher antibiotic sensivity in addition to higher diversity in lower taxa due to high hydrostatic pressure, depletion of oxygen and nutrients causing the shift of bacterial community composition according to hard environmental conditions. However, compared to those stations, resistance and diversity increased at the family level for especially station C1 in upper North Aegean Sea due to lower depth and higher nutrient input from Black Sea. On the other hand, stations B showed the highest bacterial diversity in both lower and higher taxa indicating the optimum environmental conditions supported by biogeochemical results obtained in the area.

In the present study, high resistance to ceftazidime which is beta-lactam antibiotic inhibiting cell wall synthesis indicated that cell wall composition of isolates might play a key role in preventing the entrance or binding of antibiotics to the cell. Moreover, high resistance to amikacin binding 30S ribosomal subunit and inhibiting protein synthesis and also susceptibility to chloramphenicol binding 50S ribosomal subunit and inhibiting protein synthesis suggested that the small subunit of ribosomes of isolates might be much protected against binding of antibiotics rather than large subunit.

Conclusion

As a result, this study is very crucial to understand the biogeochemical patterns in Eastern Mediterranean Sea with combination of the bacterial diversity and antibiotic resistance. The lowest susceptibility results obtained from coastal zone underlined the terrestrial and anthropogenic influence. Against antibiotics, the isolates might have more protective mechanisms for cell wall and protein synthesis.

Acknowledgements

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References

1. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59: 143-169.
2. Giovannoni S, Rappe M (2000) Evolution, diversity and molecular ecology of marine prokaryotes. In DL. Kirchman, (Ed). Microbial ecology of the oceans 47-84.

3. McIntyre A (Ed) (2010) A global census of marine microbes. In Life in the world's oceans: diversity, distribution and abundance.
4. Kouridaki I, Polymenakou PN, Tselepides A, Mandalakis M, Smith KL Jr (2010) Phylogenetic diversity of sediment bacteria from the deep Northeastern Pacific Ocean: a comparison with the deep Eastern Mediterranean Sea. Int Microbiol 13: 143-150.
5. Schauer R, Bienhold C, Ramette A, Harder J (2010) Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. ISME J 4: 159-170.
6. da Silva MAC, Cavalett A, Spinner A, Rosa DC, Jasper RB, et al. (2013) Phylogenetic identification of marine bacteria isolated from deep-sea sediments of the eastern South Atlantic Ocean 2: 127-137.
7. Kümmerer K (2009) Antibiotics in the aquatic environment--a review--part I. Chemosphere 75: 417-434.
8. Kümmerer K (2009) Antibiotics in the aquatic environment--a review--part II. Chemosphere 75: 435-441.
9. Kümmerer K (2003) Significance of antibiotics in the environment. J Antimicrob Chemother 52: 5-7.
10. Chelossi E, Vezzulli L, Milano A, Branzoni BM, Fabiano M, et al. (2003) Antibiotic resistance of benthic bacteria in fish farm and control sediments of the Western Mediterranean 219: 83-97.
11. Costanzo SD, Murby J, Bates J (2005) Ecosystem response to antibiotics entering the aquatic environment. Mar Pollut Bull 51: 218-223.
12. Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. Sci Total Environ 225: 109-118.
13. Mudryk ZJ1 (2005) Occurrence and distribution antibiotic resistance of heterotrophic bacteria isolated from a marine beach. Mar Pollut Bull 50: 80-86.
14. de Oliveira AJ, Hollnagel HC, Lima Mesquita Hde S, Fontes RF (2007) Physical, chemical and microbiological characterization of the intertidal sediments of Pereque Beach, Guarujá (SP), Brazil. Mar Pollut Bull 54: 921-927.
15. Oliveira AJFC, Franca PTR, Pinto AB (2010) Antimicrobial resistance of heterotrophic bacteria isolated from seawater and sands of recreational beaches with different organic pollution levels in southeastern Brazil: evidences of resistance dissemination 169: 335–384.
16. Perlinski P, Mudryk Z (2009) Inhibitory effect of antibiotics on the growth of heterotrophic bacteria inhabiting marine beach. Baltic Coastal Zone 13: 15-24.
17. Pianetti A, Bruscolini F, Sabatini L, Colantoni P (2004) Microbial characteristics of marine sediments in bathing area along Pesaro-Gabicce coast (Italy): a preliminary study. J Appl Microbiol 97: 682-689.
18. Ruban P, Gunaseelan C (2011) Antibiotic resistance of bacteria from Krishna Godovari Basin, Bay of Bengal, India 9: 133-136.
19. Saravanakumar R, Ronald J, Maheswari K, Ramesh U (2011) Phylogenetic identification of antimicrobial active marine bacteria from sediments of the coasts of Southeast India 1: 5-14.
20. Cardak M, Altuğ G, Ay M, Erol O (2016) Distribution of antibiotic resistance and the presence of vancomycin-resistance genes (vanA and vanB) in Enterobacteriaceae isolated from the Sea of Marmara, the Canakkale Strait and the Istanbul Strait, Turkey 45: 182-190.
21. Kimiran-Erdem A, Arslan EO, Sanli Yurudu NO, Zeybek Z, Dogruoz N, et al. (2007) Isolation and identification of enterococci from seawater samples: assessment of their resistance to antibiotics and heavy metals. Environ Monit Assess 125: 219-228.
22. Sivri N, Ozbayram G, Karatut Z (2010) Antibiotic resistance of enteric bacteria isolated from south-western Istanbul coast (Turkey) 39: 402.
23. Altug G, Cardak M, Ciftci PS, Gurun S, Saad AA, et al. (2010) Distribution and antibiotic resistance of heterotrophic and indicator bacteria in the coastal areas of Turkey, Syria and Lebanon 39: 333.
24. Akkan T, Kaya A, Dincer S (2013) Antibiotic levels and heavy metal resistance in Gram-negative bacteria isolated from seawater, Iskenderun Organized Industrial Zone 7: 10-14.
25. ASTM Standard D422-63 (2007) Standard test method for particle-size analysis of soils. ASTM International, West Conshohocken, PA.
26. Danovaro R (2009) Total organic carbon, total nitrogen and organic phosphorus in marine sediments," in Methods for the study of deep-sea sediments, their functioning and biodiversity. R. Danovaro Ed. USA: CRC Press 13-22.
27. Mincer TJ, Jensen PR, Kauffman CA, Fenical W (2002) Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. Appl Environ Microbiol 68: 5005-5011.
28. Gontang EA, Fenical W, Jensen PR (2007) Phylogenetic diversity of gram-positive bacteria cultured from marine sediments. Appl Environ Microbiol 73: 3272-3282.
29. Hayakawa M, Sadaka T, Kayiura T, Nonomura H (1991) New methods for the highly selective isolation Micromonospora and Microbispora. J Fermen Bioeng 72: 320–326.
30. Galatenko OA, Terekhova LP (1990) [Isolation of antibiotic-producing Actinomycetes from soil samples exposed to UV light]. Antibiot Khimioter 35: 6-8.
31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods 28: 2731–2739.
32. Clinical Laboratory Standards Institute (CLSI) (2012) Performance standards for antimicrobial disk susceptibility tests; approved standard – eleventh edition. Wayne, Pennsylvania, USA.
33. Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards 33: 152–155.
34. Danovaro R, Marrale D, Della-Croce N, Parodi P, Fabiano M (1999) Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling 42: 117–129.
35. Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Links between geographic location, environmental factors and microbial community composition in sediments of the Eastern Mediterranean Sea 49: 367–378.
36. Polymenakou PN, Lampadariou N, Mandalakis M, Tselepides A (2009) Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea. Syst Appl Microbiol 32: 17-26.