The occurrence of pathogenic bacteria in some ships' ballast water incoming from various marine regions to the Sea of Marmara, Turkey

Gulsen Altug [a],*, Sevan Gurun [a], Mine Cardak [b], Pelin S. Ciftci [a], Samet Kalkan [a]

[a] Istanbul University, Faculty of Fisheries, Department of Marine Biology, Istanbul, Turkey
[b] Canakkale 18 Mart University, Faculty of Fisheries, Canakkale, Turkey

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ABSTRACT

The composition and frequency of antibiotic resistance of pathogenic bacteria, the abundance of heterotrophic aerobic bacteria (HPC) and possible in-situ use of chromogenic agar were investigated in the ships' ballast water coming from different regions of the world to the Sea of Marmara, Turkey for the first time. The samples that were taken from 21 unit ships coming from various marine environments of the Southern China Sea, the Atlantic Ocean, the Mediterranean and the Black Sea to the Sea of Marmara, Turkey in 2009 and 2010 were tested. 38 bacteria species, 27 of them pathogenic bacteria belonging to 17 familia, were detected. *Vibrio cholera* was not detected in the samples. However, the presence of a high number of HPC, including a cocktail of pathogenic bacteria showed that the ships carry a potential risk for the Sea of Marmara.

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1. Introduction

Ships need ballast water and often take on a certain amount of water in order to maintain their stability and trim at the start of a voyage. The movement of ballast water between ecosystems was identified as the most important way for the transfer of aquatic non-indigenous species. Occurrences of pathogenic bacteria have potential negative effects on the ecosystem and also on human health. Terrestrial sourced pollution, over-use of living aquatic resources and habitat destruction have been considered as the three greatest menaces to the world's oceans. Apart from these pollution sources, ship ballast waters are primary carriers for global transport of *non*-indigenous marine species, harmful aquatic organisms and pathogens. These pollution sources have been identified today to be the fourth greatest danger to the world's oceans. The rates of non-indigenous species introduced to marine environments via ballast water were estimated to be 30% during the last 20–30 years (Carlton, 1985; Carlton and Geller, 1993; Ruiz et al., 1997; Cohen and Carlton, 1998).

It has been well documented that ballast water can contain protozoa, toxic dinoflagellates, metazoa, and the other microorganism including pathogenic forms (Medcof, 1975; Carlton, 1985;

Williams et al., 1988; Hallegraeff, 1998; Hallegraeff and Bolch, 1991; McCarthy et al., 1992; Carlton and Geller, 1993; McCarthy and Khambaty, 1994; Subba Rao et al., 1994; Gosselin et al., 1995; Galil and Hülsmann, 1997; Pierce et al., 1997; Chu et al., 1997; Gollasch et al., 1998; Hallegraeff, 1998; Lavoie et al., 1999; McCarthy and Crowder, 2000; Ruiz et al., 2000a,b; Drake et al., 2001).

The movements of ballast waters from one continent to another via ships cause a global distribution mechanism for pathogenic and antibiotic-resistant forms and the epidemiology of waterborne diseases affecting plants and animals (Ruiz et al., 2000b). Antibiotics are widely used for treatment of infections in the world. Resistance to clinically relevant antibiotics is common in aquatic bacteria, including potential human pathogens. Thomson et al. (2003) reported that the horizontal gene transfer of antibiotic resistance occurs within ballast tanks. Transportation of bacteria via ballast water from one region to another is increasing antibiotic resistance in marine environments.

As a result, mankind must find new antibiotics to take the place of old ones in treatment regimes. Also, the bacteria that cause nosocomial infections are often drug-resistant (Gordis, 2004).

Because of its geographical location, the Sea of Marmara is an important water route between the Mediterranean and the Black Sea. The Istanbul Strait connects the Sea of Marmara to the Black Sea and the Çanakkale Strait connects the Sea of Marmara to the Aegean Sea. The Turkish Strait System (TSS) and the Sea of Marmara are under risk due to heavy marine transportation. Annually

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* Corresponding author. Tel.: +90 212455 57 00; fax: +90 2125140379.
 E-mail address: galtug@istanbul.edu.tr (G. Altug).

number of ships which have passed through the Istanbul and Çanakkale Straits in 2010 was reported by the Turkish authorities to be 50.871 and 46.686 respectively.

In this study, with an aim to detect the transportable bacteriological risk of ships' ballast water for the Sea of Marmara, the composition and frequency of beta lactam antibiotic resistance of pathogenic bacteria, the abundance of heterotrophic aerobic bacteria were investigated in the ballast water of ships coming from various marine environments to the Sea of Marmara, Turkey.

2. Materials and methods

2.1. Sampling

The ballast waters were taken from 21 different ships which came from 12 different regions of the world such as the Southern China Sea, the Western Baltic Sea of the Atlantic Ocean, the Red Sea, the Adriatic Sea of the Mediterranean and the Black Sea. The ballast water samples were taken from the ships around the Ambarli Port, which is located on the northern shores of the Sea of Marmara. One ballast tank per ship was sampled. Three water samples were taken per tank. Seawater samples were also taken from the near vicinity of the ships. The ports of origin (Fig. 1) of those ships whose ballast waters were sampled between June 2009 and July 2010 were shown on Table 1.

2.2. Bacterial count and identification

The ballast water samples were taken by suspending sterilized glass bottles into the ballast tank under aseptic conditions. The samples were transported daily to the laboratory and serial dilutions from 10^{-5} to 10^{-12} were prepared in 9 ml amounts of sterile seawater (artificial seawater, Sigma) and were inoculated (0.2 ml) in duplicate on marine agar (Difco), and the plates were incubated for five days at 22 ± 0.1 °C (Bianchi et al., 1992). At the end of the incubating period all the colonies were counted. Different colonies were picked and restreaked several times to obtain pure cultures. The pure isolates were Gram stained and then identified using GN (Gram-negative fermenting and non-fermenting bacilli), GP (Gram-positive cocci and non-spore-forming bacilli) and BCL (Gram-positive spore-forming bacilli) cards in the automated micro identification system VITEK 2 Compact 30 (Biomereux, France). The identification cards are based on established biochemical methods and newly developed substrates. There are biochemical tests (46 tests for BCL, 43 tests for GP, 47 tests for GN) measuring carbon source utilization, enzymatic activities, inhibition, and resistance. Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "+", "−", "(−)" or "(+)". Reactions that appear in parentheses were evaluated as an indicator of weak reactions that are too close to the test threshold. The VITEK 2 system identifies the bacteria at a species level. The system can describe various qualitative levels of identification are assigned based on the numerical probability calculation. Identification confidence levels is defined to be; excellent (96%—99% probability), very good (93%—95% probability), good (89%—92%), acceptable (85%—88%), low discrimination and unidentified organism (Pincus, 2005). In this study, 99%—85% confidence levels were accepted to be a positive identification.

2.3. Antibiotic resistance tests

The percentage of antibiotic resistance bacteria in the samples was measured using antibiotic disks; Cefotaxime (CTX: 30 mg, Oxoid), Amoxycillin (AML: 10 mg, Oxoid), Ampicillin (AMP: 10 mg, Oxoid) and Imipenem (IPM: 10 mg, Oxoid) Nafcillin (NF: 1 mg, Oxoid) and Oxacillin (OX: 5 mg, Oxoid) on Nutrient Agar (Oxoid) media. The bacteria concentration of broth cultures which were adjusted to 10^5 bacteria cells per ml using McFarland turbidity standards solution of identified isolates were spread and then antibiotic discs were placed at intervals 25 mm on Nutrient Agar media. After incubation (24 h at 37 °C) the growing position of the
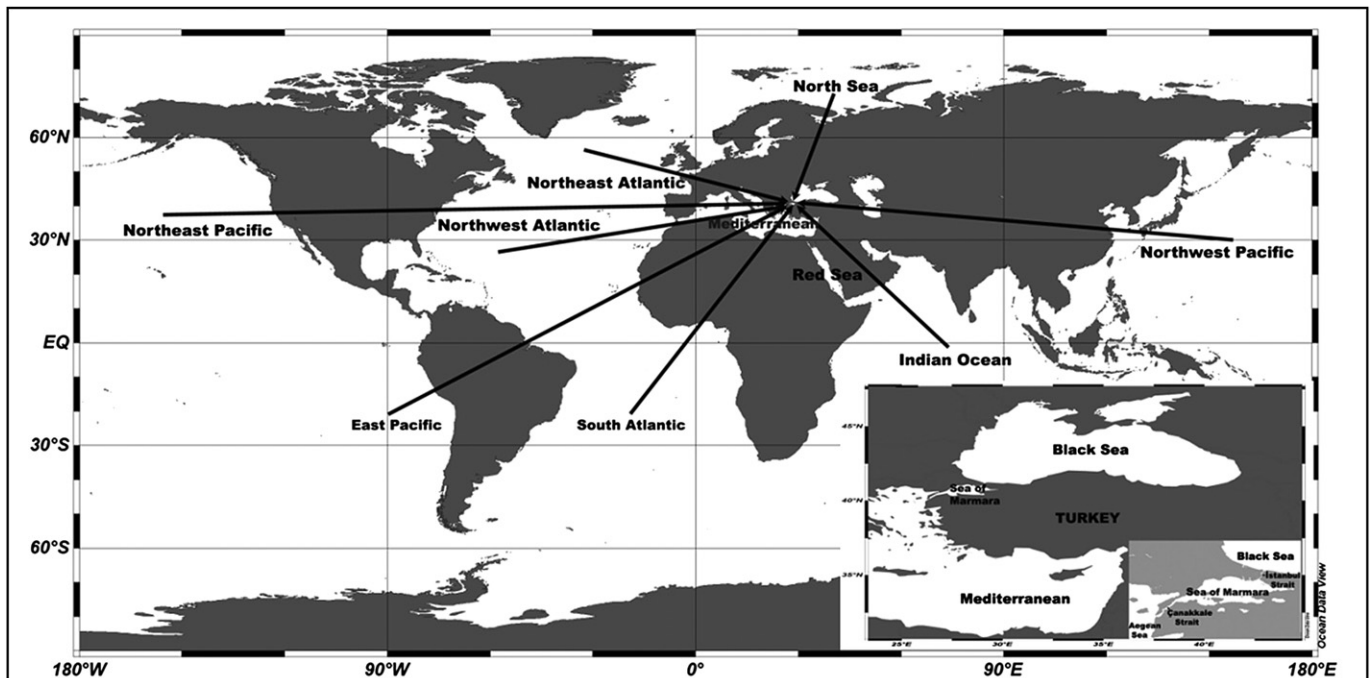

Fig. 1. Map showing the ports of the ships whose ballast waters was sampled and came from various marine regions to the Sea of Marmara, Turkey (2009—2010).