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Occurrence and distribution antibiotic resistance of heterotrophic bacteria isolated from a marine beach

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Abstract

Antibiotic resistance of heterotrophic bacteria isolated from a sandy beach in Sopot, at the Southern Baltic Sea coast was determined. The levels of resistance of bacteria to various antibiotics differed considerably. Bacteria inhabiting the middle part of the beach and the dune were most resistant; the least resistant were bacteria isolated from the sea-beach contact zone. Generally, there were no significant differences in antibiotic resistance between pigmented and non-pigmented bacteria. Bacteria isolated from the surface layer of the sand were more resistant to the tested antibiotics than bacteria from the subsurface layers. The majority of bacterial strains were resistant to 3–8 antibiotics. Bacterial resistance to antibiotics was dependent on their chemical structure.

Keywords: Southern Baltic; Sandy beach; Heterotrophic bacteria; Antibiotic resistance

1. Introduction

In the last several dozen years, antibiotic utilisation in medicine, veterinary medicine, agriculture and aquaculture has been steadily increasing; very little is however known about the amount of antibiotics entering the environment after their use (Halling-Sørensen et al., 1998; Backhaus and Grimme, 1999; Hirsch et al., 1999). The extent of antibiotic use is indicative of the selection pressure exerted on bacteria (Schwartz et al., 2003); many have developed various very efficient mechanisms to render ineffective the antibiotics used against them. Resistance can be intrinsic i.e. associated with reduced penetration of the antibiotic into the cell, or can result from active processes such as changes in the transport of antibiotics into or from the cell, from modifications of cellular target molecules, or from the production of enzymes that modify and inactivate the antibiotic (Hermansson et al., 1987).

Genes encoding those defense mechanisms are located on a bacterial chromosome or on extrachromosomal plasmids, and are transmitted to next generations (McKeon et al., 1995; Lobova et al., 2002). DNA coding for antibiotic resistance may be conjugally transferred between similar bacteria. In many cases the genetic code for antibiotic resistance is placed on so-called R-plasmids (Hirsch et al., 1999). Those plasmids can be transferred to sensitive strains of the same species, and even to different species. In water basins, transmission of R-plasmid determinants may occur in less than 1 minute and antibiotic resistance can spread rapidly among bacteria (Arvanitodou et al., 1997).

Emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used, but occurrence of antibiotic-resistant bacteria is also increasing in aquatic environments (Schwartz et al., 2003). In water bodies, concentration of antibiotics ranges from 0.01 to $9.5\,\mu g\,dm^{-3}$ in the water, and from 0.1 to $10.0\,mg\,kg^{-1}$ in the sediment (Coyne et al., 1994; Backhaus and Grimme, 1999). Antibiotic substances may strongly affect quantitative and qualitative composition of bacteriocenoses in water ecosystems and may also play a substantial role in food competition systems (Barja

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et al., 1989; Lemos et al., 1991; Sabry et al., 1997). This is why many studies have been recently carried out to determine the distribution of antibiotic-resistant bacteria in freshwater basins, estuaries, municipal drinking water and sewage waters (Nemi et al., 1983; Calomiris et al., 1984; Jones et al., 1991; Herwig et al., 1997; Ash et al., 2002; Mudryk, 2002). There are however relatively few studies on the presence of antibiotic-resistant bacteria in marine water (Nair et al., 1992; Mudryk and Skórczewski, 1998), and no such studies have been undertaken in such dynamic ecosystems as marine beaches.

Therefore, the aim of the present study was to determine the resistance to antibiotics in heterotrophic bacteria inhabiting a sandy beach at the Baltic coast.

2. Materials and methods

2.1. Study area and sampling

The material was collected from a sandy beach near Sopot, Poland, at the Southern Baltic coast (54° 27′N, 18° 33′E). The beach has a slope of 7° and is 46 m wide. It represents a dissipative beach type with longshore bars and troughs, composed of medium grain size quartz sand. The salinity of the overlying water ranges from 0.8 to 3.6. The organic content of the sand varied from 0.20% to 0.57% dry weight with lower values recorded in the middle of the beach, and higher ones towards both the dune and the waterline (Jędrzejczak, 1999). The Sopot beach is a suitable and very popular recreational area. It is frequented by holiday makers, whose density in summer reaches 30 persons per 100 m^2; about 3000 people can pass there daily (Węcławski et al., 2000).

Sand samples were taken once, in July 2001. A transect was marked along a profile formed perpendicularly to the shoreline and four sampling sites were located along this transect (Fig. 1). Site 1 was located approximately 1–1.5 m from the waterline into the water, at a depth of about 1 m; site 2 was situated at the waterline, site 3 lay halfway up the beach, at a 30 m distance from the shore, and site 4 lay in a sheltered place among the dunes, 60 m away from the shore.

Core samples were taken with a 30 × 15 cm Morduchaj-Boltowski core scoop. The sand cores were divided into two sections: 0–1 cm (surface layer) and 5–10 cm (subsurface layer), and placed in sterile glass boxes. The samples were placed on ice and immediately transported to the laboratory; the analysis commenced within 2–3 h.

2.2. Isolation of bacteria

Each of the 10.0 g sand samples was weighed aseptically and transferred to 100 cm^3 of sterile seawater for subsequent homogenisation (5 min at 23.000 rpm in the NPW120 homogeniser). The supernatant was serially diluted with sterile seawater and plated by the spread method onto ZoBell 2216 agar medium (ZB) (Rheinheimer, 1977) prepared with old brackish water, of the salinity 8. Triplicate plates from each tenfold dilution were incubated for 14 days at 20 °C. Afterwards, ≈30 bacterial colonies from each sampling site and each core section were collected at random and transferred to semisolid ZB medium. After purity control, bacteria were stored at 4 °C, with inoculation on fresh medium carried out every 3 months, and used for further studies in order to determinate their antibiotic resistance.

2.3. Determination of antibiotic resistance

Antibiotic resistance of marine bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar (Oxoid), according to the Bauer-Kirby method (Arvanitodou et al., 1997). Bacteria were multiplied on agar slants (ZB) at 20 °C. After 72 h they were washed off the slants with 5 cm^3 of sterile buffered water and adjusted to a turbidity of 4 on the Mac Farland scale, which corresponds to 10^9 bacterial cells per 1 cm^3. Subsequently, 0.2 cm^3 of bacterial suspension prepared in this way was introduced into dissolved Mueller-Hinton medium cooled to 40 °C. After mixing, the sample was poured onto Petri dishes and dried in a drier at 37 °C for 1 h. Paper discs impregnated with an antibiotic were than applied to the surface of the seeded medium. The blotting paper discs (∅13 mm) were manufactured by the Warsaw Serum and Vaccine Production Company and the Becton-Dickinson Company. The dishes were kept at 4 °C for 1 h in order to allow antibiotic diffusion from the discs into the agar medium. The dishes were than incubated at 20 °C for 24 h. After incubation, the diameter (in mm) of the areas were bacterial growth was inhibited by the antibiotics was measured. Bacteria were classified as antibiotic resistant according to the manufacturer instructions. The following seventeen clinical antibiotics, with their concentrations given in parentheses were used in antibiograms: ampicillin (AM, 10 μg), biseptol (SXT, 25 μg) chloramphenicol (CP, 30 μg), clindamicin (CM, 2 μg), cloxacillin (CL, 1 μg), doxycycline (DK, 30 μg), gentamycin (GE, 10 μg), kanamycin (KM, 30 μg), nalidixic acid (NA, 30 μg), neomycin (NM, 30 μg), nitrofurantion (NF, 200 μg), novobiocin (NB, 30 μg), penicillin (PL, 10 μg), rifampicin (RF, 10 μg), streptomycin (SM, 30 μg), tetracycline (TE, 30 μg), trimethoprim (TM, 1.25 μ g). The antibiotics were divided into six groups according to their chemical structure (β-lactams, aminoglycosides, tetracyclines, rifampicins, sulfonamides, other) (Foster, 1983). The results were used to calculate the antibiotic resistance index (ARI) for bacteria (Jones et al., 1986).