MICROBIAL POPULATION DYNAMICS IN DELTAIC AQUATIC ECOSYSTEMS – CASE STUDY ON SFANTU GHEORGHE BRANCH

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Abstract

The aquatic ecosystems – a perfect environment to propagate the characters of pathogenicity and virulence of the microorganisms, could be reservoirs of antibiotic resistance genes.
During January – June 2013, a program to investigate bacteriological water quality of St. Gheorghe branch has been created. The investigations were carried out systematically, by establishing 11 locations with anthropogenic potential risks that could influence the quality of aquatic ecosystems. The surface water and sediments samples were monthly collected and the bacteriological indicators with their antibiotic resistance profile were quantified and analyzed. The *Enterobacteriaceae* and *Pseudomonas sp.* strains with antibiotic resistance profile were identified and they were subjected to PCR technique to identify the genes encoding these resistance mechanisms. The natural resistance mechanisms to antibiotics were identified, but there was amplified a *tem* gene which encode for resistance to β-lactams.
Keywords: *bacteria, antibiotic resistance genes, Danube Delta*

Introduction

The aquatic ecosystem is the perfect environment to propagate the characters of pathogenicity and virulence of the microorganisms and it can become a reservoir of antibiotic resistance genes.
The microbiological contamination of water is a current problem with negative effects on population health, especially when the ecosystem is integrated in a protected area such as the Danube Delta. Microorganisms can penetrate all types of ecosystems, but when they acquire antibiotic resistance, the transfer of antibiotic resistance genes and the emergence of diseases are facilitated.
The aim of the experimental study was to monitor the microbiological pollution of the aquatic ecosystem in the Danube Delta (St.Gheorghe Branch) and to identify antibiotic resistant bacteria and the genes which codify the resistance mechanisms.
By exceeding the limits of productive capacity, regeneration and dilution of the aquatic ecosystems, the pollutants originating from point and diffused sources are rapidly spread by the water streams itself.[1]

In the Danube Delta are some areas where the population uses the surface water as drinking water source, without applying any treatment or disinfection processes.
Since the establishment of the Danube Delta as a Biosphere Reserve (1990), the international specialized institutions have shown their interest in developing the cooperation relations with the Danube Delta Biosphere Reserve.[2]
At national level, there is a unitary legal framework that establishes the principles for all environmental activities preservation and outlines the rules to meet the quality requirements of the surface water. The bacteriological indicators recommended to be monitored are: coliform bacteria and intestinal *Enterococcus*, but the microbiological quality of water means more than these microorganisms; it means the propagation of the bacterial characters of pathogenicity and virulence.
As a reference area for this study, the St. Gheorghe Branch was chosen as emissary, the oldest branch that carries out 22% of the water and sediment total volume. The investigation program taking into account the anthropogenic activities impact on the aquatic ecosystems.

Materials and methods

There were established 11 sampling points, considering some anthropogenic issues that could influence the aquatic ecosystem quality (Figure.1).
The investigations were performed monthly, on the selected control sections, within the surface water and sediments sampling campaigns, carried out during January – June, 2013.

Figure 1. The sampling points on the Sfantu Gheorghe Branch (S1=Iscaccea, S2=Upstream Tulcea, S3=Downstream Tulcea, S4=Nufaru, S5=Balteni, S6=Mahmudia, S7=Murighiol, S8=Uzlina, S9=Ivancea, S10=St. Gheorghe port, S11=Black Sea confluence)

The analyses were performed in Bacteriological Control Laboratory from INCD-ECOIND and also, in Microbiology Laboratory from University of Bucharest.

Sterile containers for sampling of water and sediment were used.[3]

The membrane filtration method was used to perform bacteriological analysis of surface water and multiple-tube technique for the sediment microorganism's analysis.

The coliform bacteria identification in surface water samples was performed by membrane filtration method on Chapman TTC medium and the identification of the coliform bacteria in sediment samples was performed by multiple-tube on Sodium lauryl sulphate broth.[4, 5, 6].

The *Enterococcus* in surface water samples was identified through membrane filtration method on Slanetz and Bartley medium and the *Enterococcus* in sediment samples was performed by multiple-tube method.[6, 7].

The presence of *Pseudomonas aeruginosa* was controlled only in the surface water samples and its identification was performed by membrane filtration method on Pseudomonas agar base medium.[8]

To obtain isolated colonies it was used the agarose gel and the antibioresistance was specifically performed for each bacterial class on Muller-Hinton medium by using the disc-diffusion method. Different antibiotics were used to identify the resistance of specific bacteria, as follows:

- The general antibiotics (kanamycin, nalidixic acid, tretracycline and trimethoprim) and β-lactam antibiotics (amoxicilline with clavulanic acid, ceftriaxone, ceftazidime, ampicilline and imipenem) were used for *Enterobacteriaceae*;
- The ampicillin, vancomycin, ciprofloxacin, tetracycline and gentamicin disks were used for *Enterococcus*;
- The kanamycin, ciprofloxacin and β-lactam antibiotics (imipenem, cefepim, ceftazidime and piperacillin) were tested for *Pseudomonas aeruginosa*.

To identify the genes that determine the microorganisms resistant to antibiotics, the *Enterobacteriaceae* and *Pseudomonas sp.* strains resistant to the β-lactams were selected and subjected to DNA extraction used in PCR technique.

To point out the acquired resistance genes such as ESBL type, the primers: *tem, shv, ctx-M, cmy and oxa* were used for *Enterobacteriaceae* strains and the primers: *tem, shv and pse* were used for *Pseudomonas sp.* strains.

Results and discussion

The microbiological indicators were quantitatively analyzed from surface water and sediment samples to observe the bacterial population dynamics in different environmental conditions.

The obtained results are present in Figure 2, 3, 4, 5.

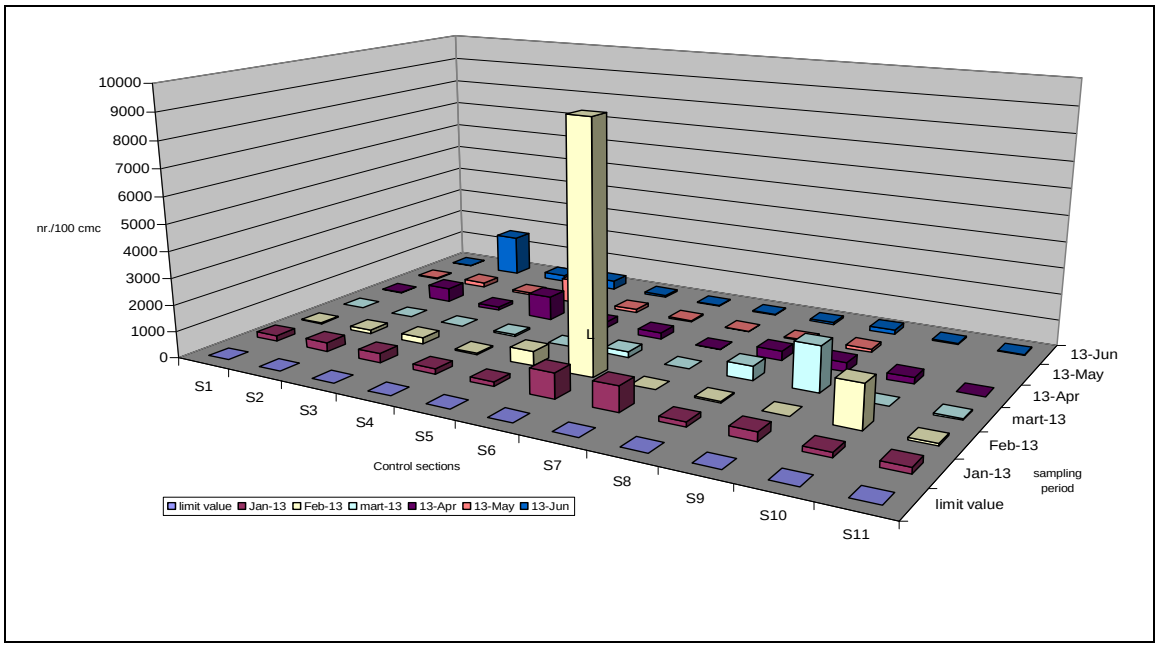

Figure 2. Spatial and temporal variation of total coliforms indicator from surface water samples taken from the 11 control sections during January – June 2013

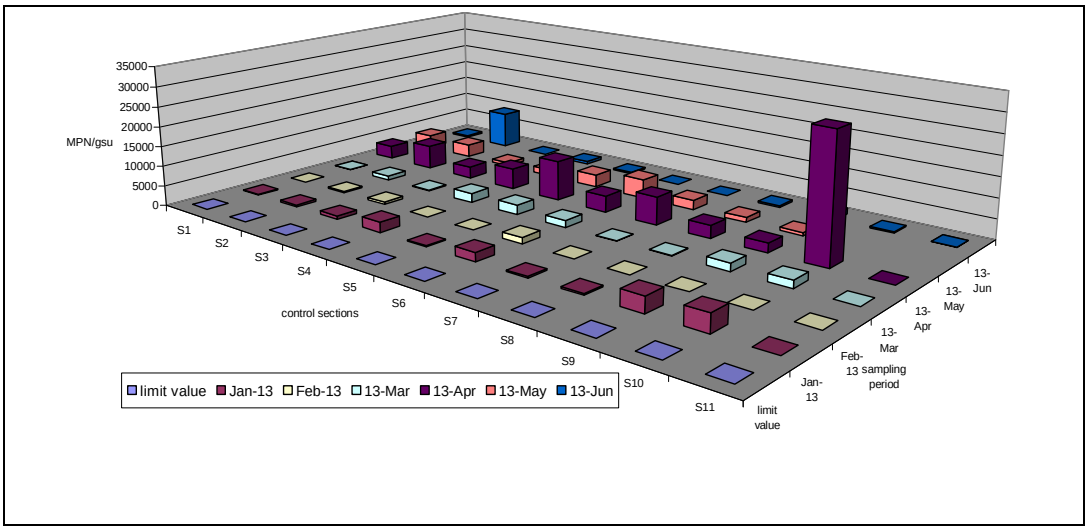

Figure 3. Spatial-temporal variation of total coliforms indicator from sediment samples taken from the 11 control sections during January– June 2013

The quantitative analysis of coliform bacteria in water and sediment samples indicated high values in February and respectively April 2013.

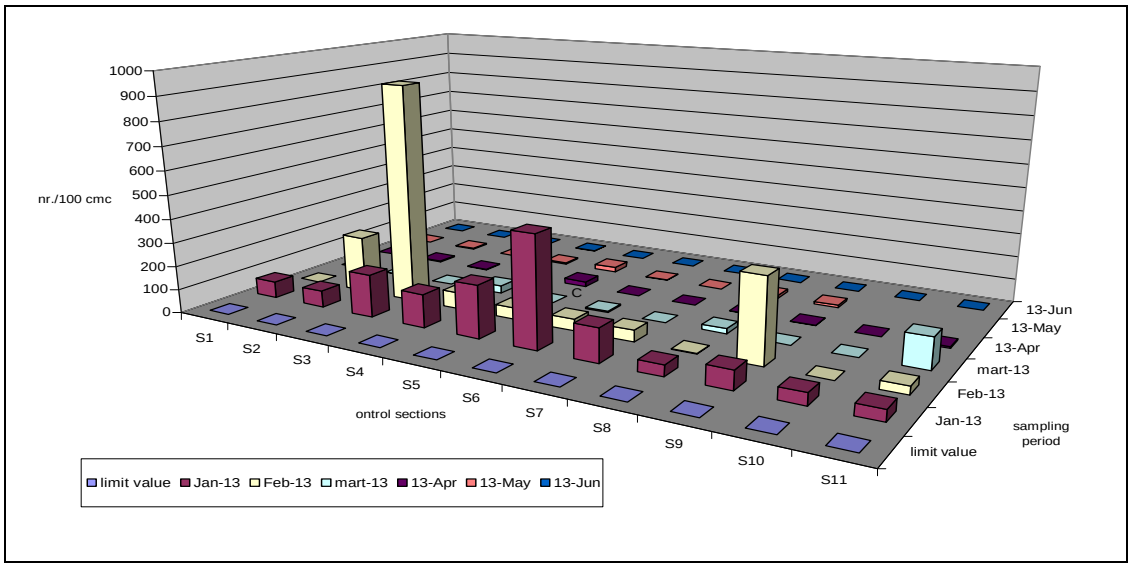

Figure 4. Spatial-temporal variation of *Enterococcus sp*. indicator from surface water samples taken from the 11 control sections during January – June 2013

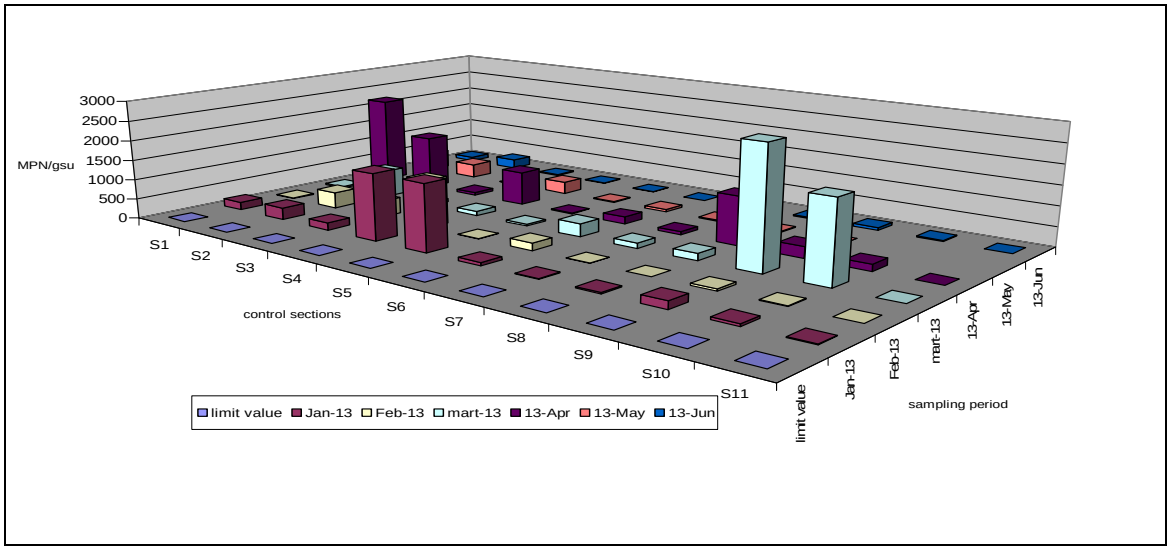

Figure 5. Spatial-temporal variation of *Enterococcus sp* indicator from sediment samples taken from the 11 control sections during January – June 2013

Enterococcus sp. recorded significantly high values in surface water samples in January and February and was found sporadically in the first three months of sampling, in the sediment samples.
Cuantification of microbiological indicators in aquatic ecosystem components is not sufficient to determine the pathogenic potential of any bacteria. Antibiotic resistant bacteria have become a constant concern throughout Europe, spreading and causing continuous occurrence of new phenotypes of resistance. Isolated strains from surface water and sediment samples were subjected to

antibiogram which indicated the presence of the mechanisms of acquired resistance.

There were identified **four β- lactams resistant bacteria**, as follows:

- *Ewingella americana* and *Pseudomonas aeruginosa* in water sample from S4 sampling point (Nufaru);
- *Pasteurella pneumotropica* in water sample from S7 sampling point (Murighiol);
- *Pseudomonas luteola* in water sample from S9 sampling point (Ivancea).

In the first half of the previous century, the production of antibiotics has been one of medicine's greatest achievements. The use of antimicrobial agents has reduced human's mortality and substantially contributed to human's increased life span. Antibiotics are used either as therapeutic or as prophylactic agents, also widely used in agricultural practices.[9]

Over time, more bacterial species have acquired increasing antibiotic resistance mechanisms and those are rapidly spreading into environment.

To emphasize the enzymatic resistance genes as ESBL type, the primers of *tem*, *shv, ctx-m, cmy* and *oxa* genes were used for the *Enterobacteriaceae* strains and the primers of *tem*, *shv* and *pse* genes for *Pseudomonas aeruginosa* strain.

The electrophoresis on agars gel showed the presence of *tem* β-lactamase at *Pseudomonas aeruginosa* strain isolated from surface water sample (Figure 6).

The natural resistance to antibiotics such as ampicilin and augumentin was demonstrated for *Ewingella americana*, *Pasteurella pneumotropica, Pseudomonas luteola* bacterial strains. It is possible that these bacteria have resistance genes which are activated only in the presence of an inducible antibiotic.

Pseudomonas aeruginosa represents a phenomenon of antibiotic resistance and demonstrates practically all known enzymatic and mutational mechanisms of bacterial resistance.[10]

It is known that *Pseudomonas spp.* has intrinsic resistance to many antibiotics and the ability to acquire gene encoding resistance determinants.

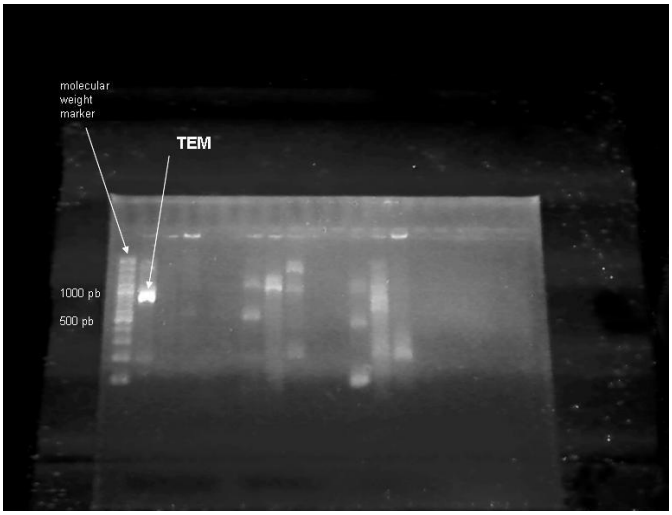

Figure 6. The *tem* resistance gene at *Pseudomonas aeruginosa* strain [water sample-S4 (Nufaru)]

The most common resistance mechanism of these pathogens is the production of β-lactamases and aminoglycoside-modifying enzymes.[11]

Conclusions

The high pathogenicity and virulence potential detected by fecal pollution indicators in the surface water indicated an impressive capacity to acquire and transmit the genetic characters that define the antibiotic resistance mechanisms. The synergism and antagonism phenomena and the resistance spectra resulting from antibiogram indicated large variety of β-lactam antibiotics to which the aquatic ecosystems microorganisms have developed resistance.
This study performed in the Danube Delta, on Sf. Gheoghe Branch emphasized both natural resistance and a *tem* gene which causes antibiotic resistance for *Pseudomonas aeruginosa*, because the β-lactam resistance became more common in aquatic environment. At this point it is important to diminish the social impact of the antibiotic resistance effect by knowing the genetic characteristics which contribute to this phenomenon. The optimal use of antibiotics will reduce antibiotic resistant bacterial strains and consequently the risk of population contamination will be reduced.

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